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Effect of irrigation water type on soil organic matter (SOM) fractions and their interactions with hydrophobic compounds

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Effect of irrigation water type on soil organic matter (SOM) fractions and their interactions with hydrophobic compounds

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List of abbreviations

^{13}C NMR - ^{13}C Nuclear Magnetic Resonance

A_{280} - specific extinction coefficient for the absorbance at 280 nm $[\mathrm{L}\ (\mathrm{g\ C})^{-1}\ \mathrm{cm}^{-1}]$

ATR-FTIR - Attenuated Total Reflectance-Fourier Transform Infrared Spectroscopy

b - dissolution domain partitioning coefficient (kg L^{-1})

C/N - the ratio between carbon and nitrogen content

C - equilibrium solution concentration (mg L^{-1})

CF1 - clay fraction < 1 μm

CF2 - clay fraction 1-2 μm

DMM - Dual-Mode Model

DOM - Dissolved Organic Matter

DRIFT - Diffuse Reflectance Infrared Fourier Transform Spectroscopy

e.g. - for instance

E4/E6 - the ratio of absorbances at 465 nm and 665 nm

FR - fractionation rest

FTIR - Fourier Transform Infrared Spectroscopy

GC-ECD - Gas Chromatography-Electron Capture Detector

GC-FID - Gas Chromatography-Flame Ionization Detector

HIX - Humification Index calculated from emission florescence spectra

HPLC-DAD - High Pressure Liquid Chromatography-Diode Array Detector

HWF - Hot Water Extractable Fraction

Kd - Langmuir affinity coefficient (L mg^{-1})

$\mathrm{Kd}_{\mathrm{OC}}$ - Langmuir affinity coefficient (L mg^{-1}) normalised to carbon content in sorbent material

K_{DM} - affinity coefficient of the hole-filling domain in DMM (L mg^{-1})
K_F - Freundlich equilibrium sorption coefficient
K-M - Kubelka-Munk intensities
$\Sigma KM_{(2920-1050)}$ - sum of selected K-M bands intensities
KM_x - Kubelka-Munk intensity of a selected band
K_{OC} - Freundlich equilibrium sorption coefficient normalised to carbon content
LF1 - specific light density fraction < 1.8 g cm^{-3}
LF2 - specific light density fraction 1.8-2.0 g cm^{-3}
N - accounts for the degree of nonlinearity in the adsorption isotherm (mg/kg) $(mg/L)^{-N}$
q - amount of solute sorbed per mass (mg kg^{-1})
Q_{DM} - capacity coefficient of the hole-filling domain in DMM model (mg kg^{-1})
Qmax - Langmuir capacity coefficient (mg kg^{-1})
Q_{OC} - Langmuir capacity coefficient (mg kg^{-1}) normalised to carbon content in sorbent material
rKM - relative Kubelka-Munk intensity
SOM - Soil Organic Matter
SPME-GC-MS - Solid Phase Microextraction-Gas Chromatography-Mass Spectrometry
TC - Total Carbon
TN - Total Nitrogen
TOC - Total Organic Carbon
WEOM - Water Extractable Organic Matter

1 Backgrounds and objectives

The deficit of water resources, growing population and the development of agriculture, and industry are the reasons of water shortage in the Middle East. The treatment of wastewater and its reuse became very important for the policy of the countries approaching to graduated replacement of freshwater allocation to agriculture by treated urban and industrial wastes. The main consumer of sludge, wastewater and treated wastewater in Middle East is the agricultural sector. The studies of wastewater irrigation mainly concerned problems related to physical changes in soil (Falkiner and Smith, 1997), salinisation of soil and water resources, the influence of wastewater on total organic carbon and nitrogen (Gerzabek et al., 2001a), soil pH (Mantovi et al., 2005), soil mineralization rates (Pascual et al., 1999) and dissolved organic matter in soil (Gigliotti et al., 1997). There are very few studies about stabilization of wastewater derived organic matter in soil and its influence on the composition of differently stabilised pools of soil organic matter (SOM).

In soils irrigated with wastewater, the changes in behaviour of commonly used pesticides were indicated, which forced the further investigation of interactions between hydrophobic substances and SOM. It has been known that wastewater irrigation can change the properties of dissolved organic matter in soil and may cause its participation in transport of heavy metals or/and xenobiotics deep in soil horizon or even to groundwater (Vulkan et al., 2002; Graber et al., 2001; Kalbitz et al., 1997). However, there are only few publications about the influence of wastewater on fate of hydrophobic compounds in different SOM fractions and about the role of these fractions in transport of hydrophobic substances in soils.

The following question should be answered:

1. Can irrigation with wastewater influence the quantity and quality of SOM?
2. Which fraction may be influenced by wastewater irrigation?
3. Is the alteration process of SOM fractions significant for sorption processes of hydrophobic substances?
4. How are the hydrophobic substances distributed among SOM fractions?
5. What kind of interaction may occur between hydrophobic substances and SOM fractions?
6. Does the aging temperature influence the distribution of hydrophobic substances in soil?
7. Does the irrigation with wastewater influence the sorption coefficients of hydrophobic substances on soil and SOM fractions?
8. What kind of sorption mechanisms ruled the sorption of model hydrophobic compounds on bulk soils and clay fractions?

2 Theoretical backgrounds

The soil organic matter (SOM) plays an important role in all processes occurring in soil and influences its biological, physical and chemical functions in environment. The residence time of pollutants in soil is dependent on the turn-over rates of SOM pools. The fractionation of SOM into the pools having different turn-over rates enables investigation of changes in organic matter which are normally not significant for the bulk soils.

2.1 Fractionation of SOM

The SOM is defined as a total organic material residing within mineral matrix (sand, silt and clay) or on its surface (Baldock and Skjemstadt, 2000). The fractionation of

SOM into different pools is based on its chemical, physical properties, or the rates of decomposition (Baldock and Skjemstadt, 2000). The term “turn-over of organic matter” expresses many processes, such as biological decomposition of organic matter by microorganisms, and chemical reactions leading to changes in SOM structures, accumulation of organic matter in soil and its stabilisation on mineral surface (Christensen, 2001). The knowledge of decomposition rates of SOM fractions is essential to assess the risk to environment in particularly how fast, and, in which form the organic pollutants can be leached to water. Various fractionation methods have been applied to study SOM nature. The choice of one of them should be preceded with the question, which chemical or physical properties of SOM are the subject of the investigation, and which land use or experimental effects on SOM considering turn-over rates of SOM fraction can be expected.

2.1.1 Classical fractionation of humic substances

The classical fractionation of humic substances results in humic acids, fulvic acids and humin (Kononova, 1961). Extensive researches in the past 40 years created much knowledge about structure and properties of humic substances and leaded to understanding of the role of humic substances in the environment. When the soil is treated with chemicals, structural and chemical changes as the result of reaction between chemicals and SOM may occur. The classical fractionation having as a product organic matter without associated mineral particles does not allow studies concerning the role of mineral matrix in formation of SOM fractions and their turn-over rates.

2.1.2 Physical fractionation

Physical fractionation methods are based on: 1) the size of organo-mineral particles, or 2) the density of organic matter. In general three principal methods are used:

a) fractionation according to particle sizes,

b) fractionation according to specific density of organic matter,

c) combination of the previous (a) and (b).

a) Particle size fractionation

The complete dispersion of aggregates with ultrasonic energy and application of different sedimentation speeds leads to separation of particles of the various sizes such as clay (< 2 μm), silt (2-2000 μm) and sand (> 2000 μm) (Schulz, 2000; Chen and Chiu, 2003; Christensen, 2001). The composition of associated organic matter depends on the size of mineral particles and their mineralogical composition (Christensen, 1992). With decreasing particle size, the content of nitrogen containing compounds (especially heterocyclic), carbohydrates, acryloaromatics, phenolics, decomposition products (hydroxyl, and carboxyl groups) was increased, in contrast to decreasing content of lignin and alkenes (Schulten and Leinweber, 2000). Clay minerals contain secondary hydrous aluminium and iron phases which can form intimate complexes with SOM and participate in stabilisation of organic molecules (Kaiser and Guggenberger, 2000). Particle-size fractionation is of great importance in investigations of soil nutrition, for instance the influence of fertilisation and land use on SOM (Desjardins et al., 2004; Christensen, 1988).

b) Density fractionation

The density fractionation is a “modern” fractionation method based on the separation of specific “light“ organic matter having density < 2 g cm^{-3}, which is not or only loosely associated to mineral matrix, and heavy fraction > 2 g cm^{-3} which is associated to mineral surface and therefore stabilized. The density separation was already performed using heavy liquids such as Na-polytungstate (Rodionov et al., 2000; Kaiser et al., 2002), halogenated organic heavy liquids such as ethanol-bromoform mixture (Shaymukhametov et al., 1984; modified by Schulz, 2004), and silica-gel solutions (Römkens et al., 1999). The silica-gel is non toxic, but its maximum density is close to 1.37 g cm^{-3} (Meijboom et al., 1995); therefore, this method did not find wide application. Recently, studies with 1.85 g cm^{-3}, 1.85-2.07 g cm^{-3} and 2.07 g cm^{-3} fractions isolated from silt particles have shown their importance in characterization of climate and land effects on SOM (Shang and Tiessen, 2000). Results of pyrolysis suggested that the heavy density fractions (> 2 g cm^{-3}) comprised highly humified organic matter, strongly associated to minerals (Schulten and Leinweber, 1999). Therefore, about 70 % of organic matter associated to clay fractions can be assigned to heavy fraction (Schulten and Leinweber, 1999). The light density fractions (< 2 g cm^{-3}) are composed of lipids, sterols and fatty acids and weakly or not associated to mineral particles; thus, they are able to rapid turn-over of organic matter. Various density fractions were frequently used to characterize the influence of cultivation on SOM (Schulz, 2000; Schulten and Leinweber, 1999).

c) Combined fractionation methods

The combination of particle-size, aggregate and density fractionations was also frequently applied. The density fractions were isolated from clay (< 2 μm), silt (2-2000 μm) and sand (> 2000 μm) particle size fractions (Schulz, 2004; Schulten and Leinweber, 1999; Shang and Tiessen, 2000). This kind of fractionation enables for: 1) classification of organic matter associated to specific particle-size fractions, and 2) studies of organic matter not associated to mineral particles which constitutes aggregate building material or is a sink of "young" organic matter (Kaiser et al., 2002; Gale et al., 2000a). The organo-mineral particles (> 2 g cm^{-3}) mainly isolated from < 2 μm particle-size fraction constitute stable part of SOM having years and even thousand years of turn-over rates. The light < 2 g cm^{-3} density fractions were characterized by high carbon concentration and rapid turn-over rates (Shang and Tiessen, 2000).

2.1.3 Other principles of SOM fractionation (WEOM and HWF)

The composition of decomposable part of SOM can be estimated from the parameters of water extractable soil organic matter. The mobile part of soil dissolved organic matter is present in the matrix pores having size > 6 µm, and is rapidly degraded by biota (Zsolnay, 2003). The decomposable part of SOM can be also characterized by the fractions obtained in artificial laboratory conditions such as cold and hot water extractable fractions (WEOM and HWF, respectively).

The $CaCl_2$ solution was used to extract WEOM from the sediment and soil samples (Corvasce et al., 2005). The WEOM is believed to contain organic matter, which has moderate to rapid turn-over rates, is present in pores having > 6 µm and 0.2-6.0 µm sizes and is available for biotic and microbial digestion (Zsolnay, 2003).

Compared to the WEOM, the hot water extractable fraction (HWF) represented a pool, which is able to rapid turn-over of organic matter, and is composed of dissociates and hydrolysates such as carbohydrates, and N-containing compounds such as amino spices and amides (Schulz, 1997; Leinweber et al., 1995). The hot water extractable carbon was proved to be an appropriate measure for the calculation of decomposable carbon pool in soil (Körschens et al., 1998). The contribution of hot water extractable carbon to SOM was at 5 %, and its content was independent from the seasonal time (Schulz, 1997; Leinweber et al., 1995). The carbon content in HWF was excellently correlated with total organic carbon in soil and carbon of microbial biomass (Schulz, 1997; Körschens et al., 1998; Chodak et al., 2003).

2.2 Qualitative and quantitative methods for SOM characterization

Elemental analyses are the basic quantitative methods used in studies of the land use effect (Rodionov et al., 2000), and of the influence of waste application on SOM (Mantovi et al., 2005). The C/N ratios were used to characterize the decomposition degree of organic matter during composting of municipal solid waste (Chefetz et al., 1996).

^{13}C NMR (^{13}C Nuclear Magnetic Resonance) and FTIR (Fourier Transform Infrared) spectroscopy belong to methods frequently used for the qualitative and quantitative characterization of SOM. The ^{13}C NMR spectroscopy has been often applied for quantitative studies of organic matter in particle-size separates and sewage sludge (Rumpel et al., 2004; Smernik et al., 2003). Disadvantages of this method are: the high cost of the single measurement, and the strong interaction of paramagnetic compounds with organic matter, in particularly iron, which shortens selectively the relaxation times of carbon species and makes impossible detection of some compounds (Gonçalves et al., 2003). Therefore, samples should be treated with HF before analysis. It was found that organic matter intimately associated to the clay particles can be dissolved during demineralisation (Schöning et al., 2005). The FTIR spectroscopy used together with ^{13}C NMR spectroscopy or as optional to ^{13}C NMR spectroscopy method (Chefetz et al., 1998; Celi et al., 1997) was applied to indicate the transformation of sewage sludge in treated wetlands, the sorption mechanisms of triazines on humic acids and to calculate the contribution of organic groups to SOM (Barber et al., 2001; Haberhauer et al., 1998; Senesi et al., 1995). The Diffuse Reflectance Infrared Fourier Transform (DRIFT) and Attenuated Total Reflectance Fourier Transform Infrared (ATR-FTIR) spectroscopy slowly displaced the classical FTIR method. To avoid the interferences in DRIFT spectra caused by mineral matrix, the destruction of the mineral component using HF (Gallé et al., 2004), or subtraction of spectrum of mineral component from the whole sample spectra is necessary (Rumpel et al., 2001; Chefetz et al., 1996; Cheshire et al., 2000). For sample preparation, it is important to obtain reproducible particle size distribution between sample and KBr. The DRIFT spectroscopy provided an opportunity for studies of mineral and organic matter surface and was applied to the qualitative investigation of DOM and the quantitative investigations of the organic groups of SOM such as: aliphatic, aromatic, hydroxyl, carboxylic, ether and amides groups (Capriel, 1997; Zaccheo et al., 2002; Celi et al., 1997; Guo and Chorover et al., 2003; Ilani et al., 2005).

The fluorescence and UV-VIS spectroscopy were frequently used to investigate the processes occurring in DOM solution. The UV-VIS parameters such as specific absorbance at 280 nm, the ratio of absorbance at 465 nm and at 665 nm as well as humification index (HIX) calculated from emission or synchronous fluorescence spectra were used to study sorption processes of DOM molecules on soil minerals, decomposition of soil-derived DOM, the influence of land use on DOM and the sorption processes of hydrophobic pollutants on humic substances (Guo and Chorover, 2003; Kalbitz et al., 2003a; Senesi et al., 1995). The HIX was also used to reveal the changes in decomposition of water extractable soil organic matter (Corvasce et al., 2005).

2.3 Role of wastewater irrigation; mobilization and immobilization of pollutants in SOM

The application of sewage sludge and wastewater to soils provides nutrients such as N, P, K and trace elements required by plants (Hallett et al., 1999), and increases plant growth and vegetables production (Al-Nakshabandi et al., 1997); however, the amount of supplied nitrogen has to be controlled due to limited assimilation capacity of plants and risk of groundwater contamination. It has been also reported that application of sewage sludge increased the mineralization of nitrogen leading to its deficient (Beltrán-Hernández et al., 1999). The wastewater irrigation resulted in slight accumulation of heavy metals in plants (Al-Nakshabandi et al., 1997).

Generally, the application of compost, of animal manure, and of sewage sludge to soil, increased carbon and nitrogen contents and reduced soil alkalinity (limed sewage sludge increased soil pH) (Hadas et al., 2004; Foley and Cooperband, 2002; Fine et al., 2002; Kacprzak and Stańczyk-Mażanek, 2003). However, in short-term experiments (3 months) the changes in SOM treated with compost were minor (Leifeld et al., 2002).

The number of studies concerning the influence of wastewater and sludge irrigation on SOM fractions is limited. The application of fresh and composted urban waste to arid soils increased content of humic substances and mineralization rates of SOM fractions (Pascual et al., 1999; Beltrán-Hernández et al., 1999). The amendment with animal manure caused an increase in lignin blocks, carboxylic groups and fatty acids mainly in silt fraction (Schulten and Leinweber, 1991; Jandl et al., 2004; Ellerbrock et al., 1999). The sand fraction of the soil was a sensitive indicator of land use changes; however, the long-term application of animal manure, wastewater and sewage sludge to soil changed also elemental composition of clay fractions (Jolivet et al., 2003; Gerzabek et al., 2001a).

Until till now, there is still a lack of knowledge of the influence of wastewater irrigation on specific light fractions. The organic matter recently added to soil, as the roots or charcoal carbon (effect of grass burning) was preferentially incorporated into < 1.6 g cm^{-3} and 1.6-1.8 g cm^{-3} light density fractions (Golchin et al., 1997; John et al., 2005; Gale et al., 2000b).

Treated wastewater and sewage sludge are regarded as a source of dissolved organic matter in soil and can participate in the transport of organic and inorganic pollutants down in soil horizon (de Jonge et al., 2002; Ashworth and Alloway, 2004). Recently, Drori et al. (2005) have reported the different sorptive capabilities of soils treated with fresh- and wastewater. The treatment of soil with municipal waste compost resulted in compositional changes of dissolved organic matter: an increase in content of hydrophilic polysaccharides molecules, and a decrease in olefinic group content (Gigliotti et al., 1997). The changes in SOM caused by sludge irrigation were dependent on the climate. The accumulation of wastewater derived DOM in soil was observed to be enhanced upon drying and wetting cycles (Seol and Lee, 2000). In soils amended with farmyard manure the increase in carbon content of hot water extractable fraction was also indicated (Schulz, 1997).

2.4 Interactions between SOM and hydrophobic compounds

“Hydrophobic compounds” are: simple aromatic compounds (e.g.: toluene, benzene and ethylbenzene), polycyclic aromatic hydrocarbons (PAHs), chlorinated hydrocarbons (e.g. HCHs) and polychlorinated hydrocarbons (e.g. PCBs). The entrance of hydrophobic substances to the soil can be via atmospheric deposition, waste application, or by spraying. In soils, the hydrophobic substances can be volatilized, biodegraded, leached, accumulated in soil biota, sequestrated in mineral pores, or adsorbed on SOM (Gerstl et al., 1997). Volatilization of hydrophobic compounds can be decreased or eliminated by the sorption on SOM (Gerstl et al., 1997). The ageing of contaminants in soil is based on their interaction with SOM, which resulted in decrease in their chemical and biological accessibility (Semple et al., 2003; Krauss and Wilcke, 2002). Sorption and diffusion are two main mechanisms of contaminants aging. The sorption of xenobiotics by soil is mainly related to carbon content and its form in soil due to preferentially adsorption of water molecules on mineral surface (Ben-Hur et al., 2003; Flores-Céspedes et al., 2002; Xing, 2001). A portion of hydrophobic substances can be rapidly adsorbed via hydrogen bonding and van der Waals forces and the other one can form covalent bonds with SOM leading to their stabilization in soil (Semple et al., 2003). PAHs have shown high affinity for the aromatic and amorphous aliphatic organic matter (Giger, 1999; Xing, 2001; Salloum et al., 2002). The humified organic matter sorbed hydrophobic substances to greater extent than the fresh cellulosic or phenolic derived organic matter (Lesan and Bhandari, 2003; Salloum et al., 2002). The triazines are able to form: 1) ionic bonds between carboxylate groups of SOM and protonated heterocyclic and amine N-atoms of triazine; 2) charge transfer (π - π) bonds between the electron-donor triazine ring and electron acceptor structural domains of HA; 3) hydrogen bonds (Senesi et al., 1995). Martin-Neto et al. (2001) specified the interaction between atrazine and SOM as electron-transfer complexes with humic acids. The contaminants can diffuse to the active structures of SOM, or can be placed in the pores of mineral fraction filled with organic matter (Semple et al., 2003). The movement of hydrophobic pollutants in soil profile can be enhanced by their reaction with soil dissolved organic matter (DOM) or by formation of derivates having lower sorption coefficients (Seol and Lee, 2000; Chiou et al., 1986). The low polarity, high hydrophibicity, amphoteric character and the high content of large molecules (> 14000 Da) can enhance the affinity of DOM for hydrophobic molecules (Raber and Kögel-Knabner, 1997). The high affinity of DOM for the hydrophobic substances leads to the formation of stable (hydrophobic molecule-DOM) complexes and can enhance the solubility of nonpolar pollutants (Raber and Kögel-Knaber, 1997). It has been shown that there is also the competition between hydrophobic molecules and DOM molecules for the active sites in SOM and therefore the sorption of hydrophobic pollutants may be decreased in the presence of DOM or in soils irrigated with wastewater (Flores-Céspedes et al., 2002; Celis et al., 1998; Drori et al., 2005). Furthermore, the DOM molecules, which can rapidly cover active sites, can then form new active places for slower hydrophobic molecules and therefore enhance their sorption on soil (Flores-Céspedes et al., 2002; Celis et al., 1998; Singh, 2003). In a contaminated wetland soil, the hot water extractable fraction, which was strongly correlated with carbon of microbial biomass, contributed about 10 % to the total ß-HCHs content. Contaminants were also found in the hot water extractable fraction extracted from the deeper soil layers. It can suggest that some components of this fraction are highly labile in soil (Schulz, 1997).

There are many publications which describe the pesticides-DOM interactions in soil due to risk of groundwater contamination. The number of studies discussing the interactions of hydrophobic molecules with stable organic matter is limited. The sorption of hydrophobic pollutants was controlled by the composition of organic matter associated to particle-size fractions. The sorption capacity of organic matter was increased with decreasing particle-size (Zhou et al., 2004; Krauss and Wilcke, 2002). Particle size of soil fractions affected: 1) transport velocity of solution, and 2) accessibility of active sites to hydrophobic molecules. The finer fractions were of particular scientific interest due to the specific large surface area and high stability in suspension (Wu et al., 2003). The silt fraction containing organic matter with great content of aromatic structures was preferable sorbent for the PAHs. More aliphatic organic matter associated to clay fractions had higher affinity for PCBs compounds characterizing by lower sorption coefficients (Krauss and Wilcke, 2002). The organic matter of coarse sand fraction had the lowest affinity either for PAHs or for PCBs (Krauss and Wilcke, 2002).

Sorption coefficients calculated from sorption isotherms were used to predict the behavior of hydrophobic pollutants in soil (Ben-Hur et al., 2003). The equilibrium partitioning model was not sufficient to describe sorption of organic pollutants in soil due to none equilibrium preferentially flow of water and macromolecules through the macropores, (Kraus and Wilcke, 2002; Gschwend and Wu, 1985; Graber et al., 1997; Gerstl et al., 1997; Enfield et al., 1989). The sorption of chemicals by natural media can be limited due to limited mass transfer in different pore sizes or due to diffusion (Brusseau et al., 1992).

Freundlich model was frequently applied to describe sorption of hydrophobic compounds to soil (Equation 1) (Ben-Hur et al., 2003; Flores-Céspedes et al., 2002):

$$q = K_F (C)^N, \tag{1}$$

where:

q	- the amount of the solute sorbed per mass (mg kg^{-1});
C	- an equilibrium solution concentration (mg L^{-1});
K_F	- the Freundlich equilibrium sorption coefficient (mg/kg) $(mg/L)^{-N}$;
N	- accounts for the degree of nonlinearity in the adsorption isotherm.

The Freundlich sorption model assumed the decreasing free energy for adsorption and infinite number of sorption sites. When $N = 1$, sorption is linear, and proportional to the equilibrium solution concentration. The Freundlich parameter N decreased when the aromaticity of organic matter was increasing (Xing, 2001). At the high temperatures the sorption coefficient K_F was decreased (Enell et al., 2005; He et al., 1995; ten Hulscher and Cornelissen, 1996).

The sorption nonlinearity expressed in Freundlich model by the N parameter was related to heterogeneous structure of SOM, and explained by the sorption Dual-Mode Model (DMM) for polymers. The assumptions to the dual mode model were formulated in the works of Zhao et al. (2001), Lesan and Bhandari (2003), Xing (2001), Leboeuf and Weber (2000) and summarized below.

The DMM assumed a simultaneously distribution of solute to the rubbery SOM which is able to rapid linear sorption (equilibrium partitioning-like model), and to the glassy

SOM domains, which are able to simultaneous linear and nonlinear, multisolutes competitive sorption, and slow non-Fickian diffusion due to occurrence of active-sites. The rubbery SOM is polymer-like organic matter, whereas the condense SOM domains were introduced as microscale active sites (holes) being hydrophobic, subnanometer scale, placed in macromolecules, between macromolecules, or between organic phase and mineral surface (Zhao et al., 2001; Xing, 2001). In the work of Leboeuf and Weber (2000) the nonlinear sorption was produced by the void places between amorphous and crystalline regions of SOM. The diffusion can occur only in the dissolution domains of SOM, which are characterized by partitioning-like sorption mechanism of hydrophobic molecules. The SOM molecules are believed to conform to spherical geometry. For the Langmuir sorption domains the affinity of the active sites (holes) for the given solute is uniform, and the number of active sites is limited.

The dual mode model consists of solid phase dissolution (partitioning) domain described by the linear term and hole-filling domain described by Langmuir term (Lu and Pignatello, 2002):

$$q = b\,C + \frac{Q_{DM}\,K_{DM}\,C}{1 + K_{DM}\,C} \tag{2}$$

Where:

q	- the amount of the solute sorbed per mass (mg kg^{-1});
C	- an equilibrium solution concentration (mg L^{-1});
b	- dissolution domain partitioning coefficient (kg L^{-1});
K_{DM}	- affinity coefficient (L mg^{-1}) of the hole-filling domain;
Q_{DM}	- capacity coefficient (mg kg^{-1}) of the hole-filling domain.

Figure 1 contains schematic isotherms for sorption by glassy polymer, which was adopted also for soils (Xia and Pignatello, 2001; Leboeuf and Weber, 1997; Zhao et al., 2001). The changes in SOM domains considering their transformation from glassy to rubbery state were first evidenced for the transition temperature (Leboeuf and Weber,

1997) and recently related to increasing solute concentration. At the low solute concentrations the sorption was linear. With increasing solute concentration the polymer-polymer interaction can be weaken, which resulted in “melting” of holes and facilitation of solute diffusion to glassy SOM (Xia and Pignatello, 2001; Braida et al., 2001). The diffusion coefficient expressed as b parameter can be increased by co-solute increasing concentrations (Zhao et al., 2001). The increased diffusion caused the majority of partitioning term over Langmuir term and resulted in enhanced linearity of sorption isotherm.

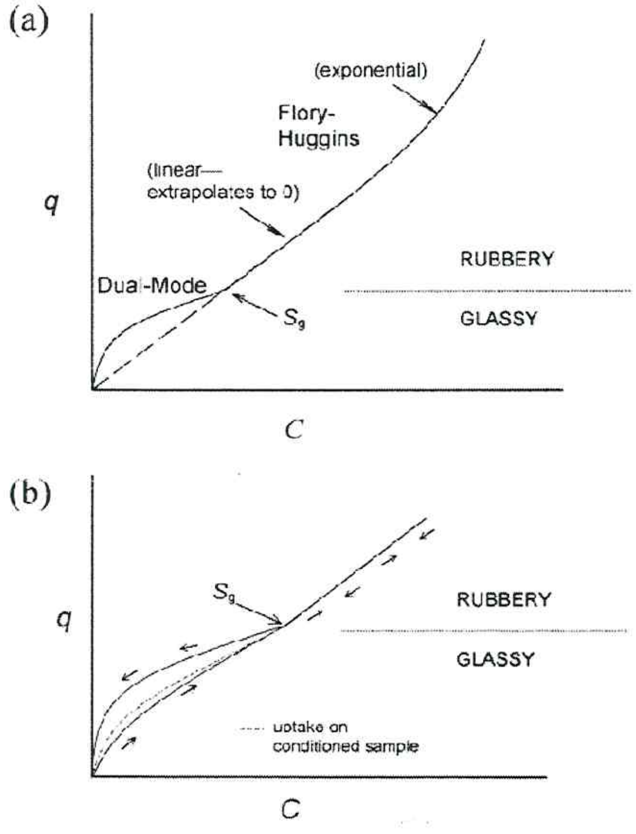

Figure 1. **Schematic of physisorption in a glassy polymer isotherm: (a) uptake isotherm showing transition from glassy to rubber state; (b) sorption-desorption hysteresis and second uptake after "conditioning" the polymer at high concentration (Adopted from Xia and Pignatello, 2001). q - the amount of the solute sorbed per mass; C - an equilibrium solution concentration**

3 Material and methods

3.1 Experimental site, soil, climate, land use

3.1.1 Site description and soil sampling procedure

This work was a part of GAPI (Groundwater Availability and the fate of Pesticide Input-Fundamentals of human health in the Middle East) project. The aim of the project was to preserve and enhance the quality of water and soil in semi-arid region for human use. Soils were samples from agricultural plots in Israel with a long history of irrigation with secondary treated wastewater.

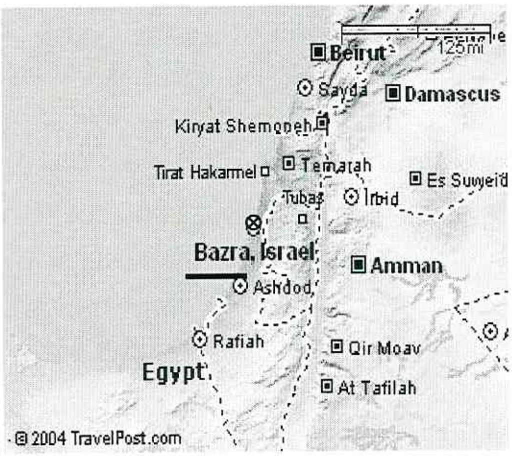

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Figure 2. The location of experimental site Bazra, Israel

Experimental site Bazra is situated at costal plain, in Israel (latitude: 32d 12m 41s; longitude: 34d 52m 29s) (Figure 2). The mean annual temperature at the site is 20 °C; mean annual precipitation is 532 mm. The region has about four rainy months between November and March.

Soil samples were collected from a citrus orchard (Bazra, Israel). The plots were irrigated for a period of more than 20 years with freshwater and secondary treated wastewater. Samples were collected using a stainless steel bucket auger from four locations (20 cm away from dripper). In each location, samples were taken from the following depths: 0-3, 3-30, 30-60, 60-90 and 90-120 cm. A composite soil sample (10 kg) was compiled from the four individual samples at each depth, air-dried, and stones and large plant material were hand-removed, large aggregates were slightly ground and passed through a 2 mm sieve.

3.1.2 Selected properties of bulk soils

The soils at Bazra site are sandy loams. Soil texture was determined with standard pipette method (Gee and Bauder, 1986) and presented together with elemental composition in Table 1.

Table 1. Selected characterization of bulk soils from different soil layers of long-term freshwater (FW) and treated wastewater (WW) irrigated plots of a citrus orchard at experimental site Bazra (Israel)

	Texture (%)					
	Clay[1]		Silt[1]		Sand[1]	
Soil depth (cm)	FW	WW	FW	WW	FW	WW
0-3	11.4	11.6	17.1	34.9	71.5	53.5
3-30	12.8	19.2	6.4	1.3	80.8	79.5
30-60	17.8	25.4	2.5	5.1	79.7	69.5
60-90	20.3	26.6	0	6.4	79.7	67.0
90-120	22.8	26.8	0	6.4	77.2	66.8
	Elemental composition					
	TOC (g kg^{-1} soil)		TN (g kg^{-1} soil)		TOC/TN	
Soil depth (cm)	FW	WW	FW	WW	FW	WW
0-3	49.9 (2.4)	59.2 (1.0)	4.97 (0.25)	5.62 (0.45)	10.0	10.6
3-30	7.3 (0.2)	6.6 (0.3)	0.77 (0.03)	0.67 (0.03)	9.5	9.7
30-60	2.9 (0.3)	5.2 (0.2)	0.29 (0.03)	0.48 (0.02)	9.9	10.6
60-90	1.9 (0.0)	4.6 (0.2)	0.38 (0.03)	0.50 (0.02)	4.9	9.3
90-120	1.3 (0.0)	3.9 (0.3)	0.25 (0.02)	0.45 (0.03)	5.3	8.7

[1] Clay, silt and sand minerals in Basra soils (texture data were produced by B. Chefetz, Hebrew University of Jerusalem, Israel)

3.1.3 Irrigation water quality

The treated wastewater used for irrigation at Bazra fields was from municipal wastewater treatment plant (Rannana, Israel). Water treatment is based on the biological transformation of organic matter contained in wastes to the carbon dioxide and is carried out by the microorganisms.

Table 2. Selected properties of freshwater (FW) and treated wastewater (WW) used for irrigation in the field plots at experimental site Bazra. The data present an average (± standard deviation) of 3-8 samples taken during 2003 (data were received from B. Chefetz, Hebrew University of Jerusalem, Israel).

	Irrigation water	
	FW	**WW**
Electrical conductivity (dS m^{-1})	1.2 ± 0.1	1.4 ± 0.03
pH	7.3 ± 0.2	7.9 ± 0.1
DOC (mg L^{-1})	NA	32.0 ± 22.6
N-NO_3^- (cmol L^{-1})	1.0 ± 0.1	0.02 ± 0.01
N-NH_4^+ (cmol L^{-1})	NA	0.06 ± 0.04
P (cmol L^{-1})	NA	0.02 ± 0.00
Cl^- (cmol L^{-1})	0.50 ± 0.01	0.60 ± 0.01
HCO_3^- (cmol L^{-1})	0.50 ± 0.06	0.60 ± 0.07
Na^+ (cmol L^{-1})	0.40 ± 0.01	0.60 ± 0.04
Ca^{2+} and Mg^{2+} (cmol L^{-1})	0.35 ± 0.10	0.35 ± 0.01
K^+ (cmol L^{-1})	0.08 ± 0.01	0.06 ± 0.01

NA-not available, values were not detectable

Irrigation water samples were taken in pre-cleaned 1 L amber glass bottles and were kept refrigerated immediately after sampling. Before analyses the samples were first filtered through a filter paper to eliminate the suspended matter and then passed through the 0.45 μm membrane filter. The samples were frozen until analysis. The fresh- and secondary treated wastewater characteristics are given in Table 2.

3.2 Fractionation of SOM

3.2.1 Hot water extraction

The hot water extraction was performed according to Schulz and Körschens (1998) and Schulz (2004). The 10 g of soil sample were placed into a flat bottom flask and boiled in deionized water (2 : 5; w : v) under reflux conditions for 1 hour. After cooling down to room temperature extracts were centrifuged at 1780 x g (4000 rpm) for 10 min (centrifuge, Universal 32R, A. Hettich, Tuttlingen, Germany) and filtered with 0.45 μm membrane RC filters (regenerated cellulose, reinforced with non-woven cellulose, Sartorius AG, Göttigen, Germany).

3.2.2 Granulo-densimetric fractionation of SOM

The SOM fractionation was performed according to differences in particle-size and specific densities (Shaymukhametov et al., 1984 modified by Schulz, 2004). Soil samples (20 g) were placed into centrifugation tubes (100 mL, without screw cup), and 70 mL of deionized water was added and carefully mixed. Each soil sample had four replications. Fractionation procedure had three phases:

- separation of plant's residues by flotation in water phase and centrifugation at 440 x g (2000 rpm) for 10 minutes (centrifuge, Universal 32R, A. Hettich, Tuttlingen, Germany), and transfer of the water phase to pre-weighted evaporating cup,
- separation of clay-size particles (< 2 μm) using ultrasonic energy and the following application of different sedimentation speeds to isolate < 1 μm particles from 1-2 μm particles,
- density fractionation of the fractionation rest remained from particle-size fractionation (> 2 μm), which is based on the partition of density separates in heavy

liquid mixture (bromoform/ethanol) and isolation of two subfractions having specific densities of < 1.8 g cm^{-3}and of 1.8-2.0 g cm^{-3}.

Isolated fractions (clay fractions, density fractions and fractionation rest) were transferred from centrifugation tubes to pre-weighted evaporating cups using alcohol, and were placed on the water bath (GFL, Großburgwedel, Germany) and weighted after drying for the calculation of mass balances (Appendix 1).

In Chapter 4, clay and density fractions were named:

CF1 - clay fraction < 1 µm,
CF2 - clay fraction 1-2 µm,
LF1 - specific light density fraction < 1.8 g cm^{-3},
LF2 - specific light density fraction 1.8-2 g cm^{-3},
FR - fractionation rest.

Particle-size fractionation

The procedure was repeated 15 times to avoid overheating of the sample.

The clay-size particles (< 2 µm) were separated by an application of ultrasonic energy of 300 J mL^{-1} (1 min.) using probe type ultrasonic disintegrator (Schmidt et al., 1999) (Model UP 200S, 24 Hz, Dr. Hielscher GmbH, Teltow, Germany) equipped with 7 mm diameter steel sonotrode. After application of ultrasonic energy the sample was centrifuged at 110 x g (1000 rpm) for 3 min. The settling phase (fractionation rest) was next used in density fractionation for the isolation of the light density fractions, and obtained floating phase (< 2 µm fraction) was transferred to another centrifugation tube and centrifuged at 440 x g (2000 rpm) for 10 min to separate < 1 µm fraction (supernatant) from 1-2 µm fraction (sediment). The supernatant containing < 1 µm fraction was transferred to the another centrifugation tube and after adding of 1 mL $MgSO_4$ centrifuged at 1410 x g (4000 rpm) for 10 min to decant the rest of water.

Density fractionation

The density mixture 2.0 g cm^{-3} was produced by mixing 345 mL of ethanol (Riedel de Haën, Honeywell Specialty Chemicals Seelze GmbH, Seelze, Germany) and 655 mL of bromoform (bromoform stabilised, 96 %, Acros Organics, Geel, Belgium). The density mixture < 1.8 g cm^{-3} was produced by mixing 454 mL of bromoform and 546 mL of ethanol. For density check, the solutions were weighted in 10 mL graduated flasks (Brand GmbH, Wertheim, Germany), and the density tolerance was fixed at ± 0.05 g cm^{-3}.

The fractionation rest from particle-size fractionation was transferred to 50 mL plastic centrifugation tube (VWR supplier, Centrifuge Tubes with Plug Caps, Polypropylene, Sterile) using ethanol. Sample was shaken and centrifuged at 1370 x g (3500 rpm) for 10 minutes to remove the rest of water (supernatant). The procedure was repeated one time. Afterwards, the 20 mL of the density mixture < 2 g cm^{-3} was added to fractionation rest, horizontally shaken for ten minutes, and centrifuged at 1000 x g (3000 rpm) for 15 minutes. The floating phase containing < 2 g cm^{-3} fraction was poured into separating funnel filled with 50 mL of water. The addition of density mixture was repeated up to six times (the supernatant had to be clear).

After the separation of < 2 g cm^{-3} fraction the fractionation rest was 3 times washed by shaking for 5 minutes with 10 mL ethanol solution, centrifuging at speed of 1000 x g (3000 rpm) for 10 minutes. The rest of ethanol was wasted.

Water, density mixture, and density fraction < 2 g cm^{-3} collected in the separation funnel were carefully shaken and left overnight. The bromoform phase settled at the bottom was decanted, and wasted. The solid phase was collected in centrifugation tube and three times washed in 10 mL of ethanol (as described above) to remove the rest of bromoform.

The 20 mL of 1.8 g cm^{-3} density mixture was added to the centrifugation tube, shaken for 5 minutes and centrifuged at 1000 x g (3000 rpm) for 5 minutes. The floating phase (< 1.8 g cm^{-3}) was poured into the separation funnel filled with 50 mL of water. The procedure was repeated three times. After 24 hours, the solid phase containing < 1.8 g cm^{-3} fraction was collected into the centrifugation tube, washed three times with 10 mL ethanol (as described above). The settling phase containing 1.8-2.0 g cm^{-3} fraction was three times washed with ethanol (as described above).

3.3 Removal of organic matter from soil and clay fractions

3.3.1 Removal of organic matter from soil and clay fractions using H_2O_2

Removal of SOM for **DRIFT analyses**, was performed by treating 1 g of bulk soil sample with 70 mL of 30 % H_2O_2 (Merck-Schuchardt, Hohenbrunn, Germany) for 0.5 hour at 20 °C and next at 80 °C for 3 days (Mikutta et al., 2005). The organic matter from a 200 mg of clay fraction (< 1 μm or 1-2 μm particle size) was removed using 250 mL of 30 % H_2O_2, first at a temperature of 20 °C for 0.5 hour and next at a temperature of 80 °C for 5 days. The samples were washed with distillate water and tested for organic carbon content. The removal of organic matter was at about 90 % of initial carbon concentration in sample.

3.3.2 Removal of organic matter from soil and clay fractions using NaOCl

The conditions of the procedure enable to avoid dissolution of Al secondary phases, which are important for **sorption processes** (Mikutta et al., 2005). Organic matter in soil samples was destroyed using 6 % NaOCl (Merck KGaA, Darmstadt, Germany), at a soil-solution ratio of 1:50 (w/v), at pH 8. The samples were agitated for 6 hours at 25 °C and centrifuged at 1780 x g (4000 rpm) for 30 minutes (Kaiser et al., 2002).

Afterwards, supernatant was removed. The procedure was repeated five times for the bulk soil samples, and seven times for clay particle-size fractions. The samples were washed with deionized water to remove salts. This procedure leaded to removal approximately from 96 to 99 % of organic matter (TOC) in soil and clay samples.

3.4 Analytical methods

3.4.1 Elemental analyses

Total carbon (TC) and total nitrogen (TN) of bulk soils (Table 1; Chapter 3.1.2) and soil fractions (Table 9; Chapter 4.1.3) were determined by dry combustion at 1150 °C in an oxygen stream according to DIN/ISO 13878 using Vario EL C/N/S analyzer (ELEMENTAR, Hanau, Germany). The TN and TC contents in the samples were calculated on the dry matter basis. The dry matter of bulk soils and their fractions was measured using Halogen Moisture Analyzer HB43 (Mettler-Toledo GmbH, Giessen, Germany). Total organic carbon (TOC) was calculated on the difference between TC and total inorganic carbon (TIC) (C-mat 550, Ströhlein Instruments, Viersen, Germany). The TIC in soil samples was measured by treating a soil with phosphoric acid (15 %) to destroy carbonates, and releasing carbon dioxide was detected and quantified using Non-Dispersive Infrared (NDIR) detector.

Nitrogen and organic carbon in hot water extractable fraction were measured using an elemental analyzer for liquid samples (Multi N/C 3000, Analytik Jena, Jena, Germany). The carbon was oxidized in the oxygen stream to carbon dioxide at 450 °C in the presence of thermocatalyst (CeO_2) and measured in Non-Dispersive Infrared (NDIR) detector. The inorganic carbon was gained by acidification with phosphoric acid (15 %). The resulted CO_2 was measured by NDIR detector. The TOC was determined using the difference method by subtracting TIC from TC. In the thermocatalytic reaction the nitrogen oxide was formed, and detected by NDIR detector.

3.4.2 Spectroscopic methods

3.4.2.1 UV-VIS spectroscopy measurements

The UV-VIS absorbances of the HWF samples were measured at pH 7 in 1 cm cuvette (Starna GmbH, Pfungstadt, Germany) by Ultrospec 300 spectrometer (Pharmacia Biotech Ltd., Cambridge, England). The ratio of absorbances at 465 nm and at 665 nm (E4/E6) was calculated and used as an indicator of polycondensation degree of organic matter in HWF (Appendix 2) (Chen et al., 1977). The specific extinction coefficient A_{280} for the absorbance at 280 nm correlating with aromatic structure content was calculated and expressed in L $(g\ C)^{-1}\ cm^{-1}$ (Appendix 2) (Kalbitz et al., 2003b).

3.4.2.2 Fluorescence spectroscopy

Fluorescence emission spectra of acidified hot water extracts (pH 2) were recorded using a Carry Eclipse fluorescence spectrometer (Varian Ltd., Victoria, Australia). The excitation wavelength was recorded in the range from 300 nm to 500 nm at a fixed excitation wavelength of 254 nm (Corvasce et al., 2005). A humification index (HIX) was calculated from emission fluorescence spectra as the ratio of the spectral area between 435-480 nm and 300-345 nm (average of 3 replicates) (Appendix 2) (Zsolnay et al., 1999). When UV-VIS absorbance at 254 nm was greater than 0.3 cm^{-1}, the samples were diluted to minimize absorbance error (Zsolnay, 2003).

3.4.2.3 Diffuse Reflectance Fourier Transform (DRIFT) spectroscopy

Spectra of solid fractions were recorded using a Perkin Elmer Spectrum One FTIR spectrometer equipped with DRIFT (Diffuse Reflectance Infrared Fourier Transform) accessory.

Six milligrams of bulk soil or solid SOM fraction (clay or density fractions) were finely mixed with 194 mg KBr (FT grade, Merck KGaA, Damstadt, Germany) in a stainless-steel ball mill (Retsch GmbH and Co. KG, Haan, Germany) at a vibration frequency of

30 Hz for 5 minutes. Samples were transferred into a micro-cup sample holder and smoothed with a powder hopper (both from Perkin Elmer, Beaconsfield, England). Absorbance spectra (100 scans) were recorded in a 500 to 4000 cm^{-1} wavenumber range at 4 cm^{-1} resolution using a Spectrum One FTIR spectrometer equipped with a diffuse reflectance accessory (Perkin Elmer, Beaconsfield, England). The spectrum of pure KBr (background spectrum) prepared as described above was subtracted from the spectra of soil or SOM fractions. All spectra were subjected to linear baseline correction using 4000 cm^{-1}, 2000 cm^{-1}, 860 cm^{-1} as zero absorbance points (Chefetz et al., 1996).

Processing of the DRIFT spectra of bulk soils and clay fractions

Spectra of the organic matter component of bulk soils and clay fractions were obtained by subtracting the spectra of the mineral component (Chapter 3.3.1) from the spectra of the whole soil or clay fraction applying 6.0 Version of program GRAMS/32 6.0 (Galactic Co., Salem, USA) (Zaccheo et al., 2002). The spectra-subtraction method is based on an algorithm called "dewiggle" (Friese and Banerjee, 1992), now implemented in GRAMS/32 6.0 version software. A detailed description of the method is given in studies of Zaccheo et al. (2002). The spectra of bulk soils and clay fractions were not quantified and therefore remained in absorbance units.

Processing of the DRIFT spectra of < 1.8 g cm^{-3} fraction

The DRIFT spectra of < 1.8 g cm^{-3} fraction (Figure 3) were converted to Kubelka-Munk units. The DRIFT peak's intensities of < 1.8 g cm^{-3} fraction spectra were normalized using equation 3 (Haberhauer et al., 1998; Haberhauer and Gerzabek, 1999):

$$rKM_x(\%) = \frac{KM_x}{\sum KM_{(2920-1050)}} 100 \qquad (3)$$

rKM - the relative Kubelka-Munk intensity;

KM_x - the Kubelka-Munk intensity of a selected band ;

$\Sigma KM_{(2920-1050)}$ - the sum of selected bands intensities at 2920, 1720, 1670, 1610, 1510, 1450, 1420, 1370, 1240 and 1050 cm^{-1};

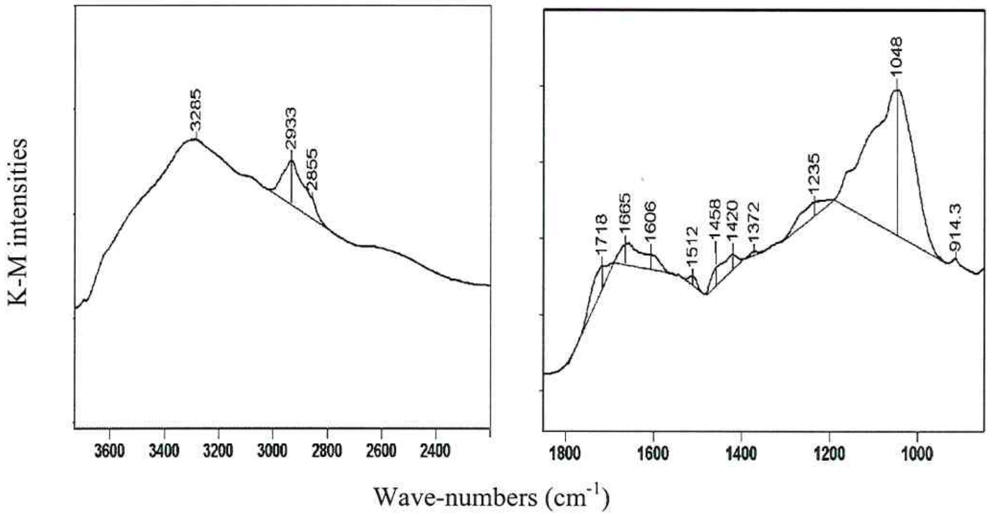
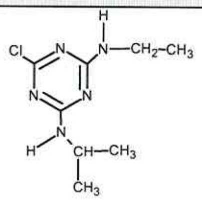
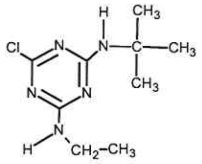
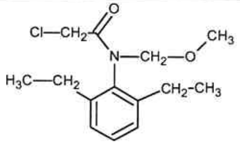
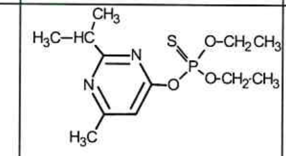
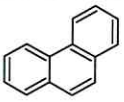


Figure 3. **Example of DRIFT spectra (Kubelka-Munk units) of a specific “light” organic matter fraction (density < 1.8 g cm^{-3}) from 3-30 cm soil layer of treated wastewater irrigated plot of experimental site Bazra with marked baselines used to determine the band intensities**

3.5 Characterization of model compounds and preparation of stock solutions

To hydrophobic compounds used for the laboratory experiments, and named in this work “model compounds” belong: atrazine, terbuthylazine, alachlor, dizinon and phenanthrene. They were found in soil and groundwater in Israel. Information about mobility, degradation time and volatilization of model compounds were adopted from: “FAO: Assessing soil contamination A reference manual” (FAO, 2000), “WHO Guidelines for Drinking-water Quality, Health criteria and other supporting information" (World Health Organization, Geneva, 2003), internet pages of U.S. Environmental Protection Agency and WHO/FAO data sheets on pesticides. Chemical characteristic of hydrophobic compounds is given in table 3.

Table 3. Chemical and structural characteristic of model compounds (phen. - phenanthrene, atrazine, terbut. - terbuthylazine, alachlor, diazinon)

Name	Structure	Formula	IUPAC name	logK$_{OW}$^{a}	Water solubility mg L^{-1}
Atrazine		$C_8H_{14}ClN_5$	6-chloro-*N*^{2}-ethyl-*N*^{4}-isopropyl-1,3,5-triazine-2,4-diamine	2.75^b	33 (20 °C)^b
Terbut.		$C_9H_{16}ClN_5$	*N*^{2}-*tert*-butyl-6-chloro-*N*^{4}-ethyl-1,3,5-triazine-2,4-diamine	3.04^b	8.5 (pH 7, 20 °C)^b
Alachlor		$C_{14}H_{20}ClNO_2$	2-chloro-2′,6′-diethyl-*N*-methoxymethyl-acetanilide	2.63 and 3.53^c	242 (25 °C)^c
Diazinon		$C_{12}H_{21}N_2O_3$ PS	*O*,*O*-diethyl *O*-2-isopropyl-6-methylpyrimidin-4-yl phosphorothioate	3.3^d	40^e; 60^f (20 °C)
Phen.		$C_{14}H_{10}$	phenanthrene-3,4-diol	4.46^g	1.13^g

[a] logK$_{OW}$ - octanol-water partitioning coefficient
[b] Chefetz et al., 2004
[c] US Environmental Protection Agency, technical fact sheet on: alachlor
[d] US Environmental Protection Agency: Environmental risk and assessment for diazinon
[e] FAO: Assessing soil contamination A reference manual
[f] International Chemical Safety Cards
[g] Georgi, 1998

Atrazine belongs to the group of chloro-triazine herbicides used for the control of broadleaf weeds and some grassy weeds. Atrazine is used on: corn, sorghum, sugarcane, wheat, guava, macadamia nuts, hay, pasture, summer fallow, forestry or woodlands, conifers, woody ornamentals, Christmas trees and sod. This pesticide itself has low acute toxicity, but the chlorinated metabolites of atrazine formed in animal tissues, in soil and water are highly toxic. A half life-time of 50 days for atrazine (laboratory conditions) was reported. In alkaline environment or in presence of humic materials (working as catalyst), atrazine was rapidly hydrolysed. Atrazin can be weekly adsorbed on soil; therefore, it is of high or medium mobility.

Terbuthylazine belongs to group of chloro-triazine herbicides and has the same applications as atrazine, but higher than its persistence in soil. In soil terbuthylazine can be mineralised by bacteria and fungi to nitrogen containing derivates and carbon dioxide. The degradation time of terbuthylazine can be highly influenced by temperature, moisture levels, microbial activity, pH, and aeration of soil. The adsorption of terbuthylazine in soil occurred in the time of two hours and was dependent on organic matter content in soil. Terbuthylazine has a half life-time of 25 days (field conditions) (Ma et al., 2004). The mobility of terbuthylazine in soil is lower than that of atrazine. A low volatility of this pesticide was also reported.

Alachlor belongs to aniline herbicide group and is slightly toxic. This herbicide is to control grasses and broadleaf weeds in filed corn, soybeans and peanuts. Alachlor can be evaporated, biodegradated or photodegradated in soil. The half life-time of alachlor is from 6.5 to 8 days in field conditions (Aga and Thurman, 2001).

Diazinon belongs to organophosphate insecticide being slightly or moderately toxic in dependence on the formulation. It is used to control cockroaches, silverfish, ants, and fleas in residential, non-food buildings and many leaf eating garden insects. Diazinon is not strongly bond to soil and is more stable in alkaline environments than at neutral or at acidic pH. Diazinon half life-time is of term from 1.2 to 5 weeks (non-sterile soil), and from 6.5 to 12.5 weeks (sterile soil). Diazinon in soil is not evaporated and is supposed to be degradated by the cometabolic attack in rizosphere in the presence of root exudates (Hsu and Bartha, 1979).

Phenanthrene belongs to the group of polycyclic aromatic hydrocarbons appearing in environment as products of incomplete combustion of fossil fuels and wood. Phenanthrene is almost insoluble in water and is rapidly adsorbed on SOM. Oxidation and volatilization of phenanthrene are not significant. Phenanthrene can be degradated in root zone by the bacteria communities (e.g. Pseudomonas bacteria in presence of biosurfactant-producing bacteria) (Dean et al., 2001).

For laboratory experiments, the **stock solutions** of the model compounds were prepared from: atrazine (99 % purity), diazinon (97.6 % purity), alachlor (99.0 % purity), terbuthylazine (99.0 %), and phenanthrene (99.5 %) (all from Dr Ehrenstorfer GmbH, Augsburg, Germany) either in acetonitryl (HPLC gradient grade) (for HPLC and GC-FID measurements) or in methanol (min. 99.9 %) (Solid Phase Microextraction-Gas Chromatography-Mass Spectrometry; SPME-GC-MS measurements) (both solvents were from Riedel de Haën, Honeywell Specialty Chemicals Seelze GmbH, Seelze, Germany). The **stock solutions** contained: a) phenanthrene, alachlor and diazinon, or b) atrazine and terbuthylazine. The concentration of each compound in the solution was at 100 mg L^{-1}.

3.6 Analysis of model compounds

3.6.1 Extraction of model compounds

The extraction of model compounds from soils and SOM fractions was carried out using Accelerate Solvent Extractor (ASE 200, Dionex, Sunnyvale, USA). The bulk soil (1 g) or soil fraction (0.02 g for plant material; 0.2 g for 1-2 μm fraction; 0.5 g for

< 1 µm fraction; 0.5 g for 1.8-2.0 g cm^{-3} fraction; 0.05 g for < 1.8 g cm^{-3} fraction; 5 g for fractionation rest) was placed in 11 mL stainless-steel extraction cell filled previously with filter (to avoid sample breaking through) and sand (washed and ignited, J.T. Baker, Deventer, Holland). The remaining space in the cell was filled with sand. Dispersion in sand keeps the sample particle from agglomerating when heated to ensure efficient extraction (Dionex Corporation, 1998). The extraction conditions are given in Table 4.

Table 4. Extraction conditions for model compounds using ASE equipment (Böhme, 2000)

	Atrazine, Terbuthylazine	Alachlor, Diazinon, Phenanthrene
Solutions (Vol:Vol)	acetone : dichloromethane (1 : 1)	n-hexane : acetone (1 : 1)
Heat	7 min	5 min
Static	5 min	5 min
Flush	60 vol	60 vol
Purge	120 s	60 s
Pressure	100 bar	100 bar
Temperature	140 °C	100 °C

The ASE extracts were evaporated at the room temperature under gentle stream of dry nitrogen to nearly dryness. The extract of model compounds was transferred to the chromatographic vial. The samples containing atrazine and terbuthylazine were diluted in defined value of acetonitrile and then the content of model compounds was determined by High Pressure Liquid Chromatography equipped with Diode-Array Detector (HPLC-DAD). The samples containing alachlor, diazinon and phenanthrene were diluted in isooctane and the content of model compounds was determined by Gas Chromatography equipped with Flame Ionization (GC-FID) and Electron Capture (GC-ECD) detectors.

3.6.2 Chromatographic methods for quantification

3.6.2.1 HPLC equipped with diode-array detector

Determination of atrazine and terbuthylazine in ASE extracts was carried out with the Agilent 1100 liquid chromatograph (Waldbronn, Germany) equipped with a binary pump (flow: 0.4 mL min^{-1}), autosamler (5 µl), a vacuum degasser, column termostated to 25 °C (124 mm x 2 mm, UltraSep ES PAH, SepServ, Berlin, Germany). The solvent mixture used for HPLC measurements was: water : acetonitrile (65:35, v:v). Triazines content was detected with diode-array detector fixed at 225 nm (atrazine retention time was at 4.16 min and terbuthylazine retention time was at 6.47 min). The calibration curves were determined using external standards, and compounds concentrations were calculated from peaks area. System was controlled with ChemStation software (Agilent, USA).

3.6.2.2 Gas Chromatography equipped with Flame Ionization (FID) and Electron Capture (ECD) detectors

A gas chromatography was used to determine contents of alachlor, diazinon and phenanthrene. The conditions are given in Table 5.

Gas chromatograph (HP 6890 series, GC system, Agilent, USA) was equipped with injector (1 µL) (7683 Series Injector, Agilent, USA), capillary column (HP 19091A-002; 0.2 mm x 25 m x 0.11 µm; max temp.: 325 °C, methyl siloxane), electron capture (ECD) and flame ionization (FID) detectors (Agilent, USA). The calibration curves were determined using external standards, and concentrations were calculated from peak areas. System was controlled with ChemStation software (Agilent, USA).

Table 5. Conditions for identification and quantification of model compounds (alachlor, diazinon and phenanthrene) using GC-FID and GC-ECD systems

	Conditions
Oven	• 80 °C initial temp. (2 min) • 325 °C maximum temp., 3 min equilibrate on time • run time 23.25 min
Front inlet	• 190 °C initial temp., 125.5 kPa • 45.2 mL min^{-1} purge flow (0.75 min.) • 47.8 mL min^{-1} total flow, nitrogen carrying gas
Column	• 0.8 mL min^{-1} initial flow; 125 kPa pressure
Front detector ECD	• 250 °C temp. • 0.1 mL min^{-1} anode purge flow • 0.5 mL min^{-1} nitrogen flow
Back detector FID	• 300 °C temp. • 40 mL min^{-1} hydrogen flow • 280 mL min^{-1} air flow • 45 mL min^{-1} makeup flow (nitrogen)

3.6.2.3 Solid Phase Microextraction-Gas Chromatography-Mass Spectrometry

The SPME-GC-MS system (GC system, HP 6890 series, Agilent, USA; 5973 Mass Selective Detector) was equipped with an automatic headspace sampling system (CombiPAL autosampler equipped with a SPME adapter, both from CTC Analytics, Zwingen, Swiss), capillary column (Agilent 19091S-433, 0.25 mm x 30 m x 0.25 µm, max. temp. 350 °C) and mass spectrometer. Syringe holder, syringe and PDMS SPME fiber (100 µm), were from Supelco (Bellefonte, PA, USA). The system conditions are presented in Table 6.

Autosampler software was from CTC Analytics (Zwingen, Switerland) and gas chromatograph was controlled with ChemStation software (Agilent, USA). Mass-to-charge ratios (m/z) for model substances were: phenanthrene (178), atrazine (215), terbuthylazine (214), alachlor (160), diazinon (179).

Table 6. Conditions for identification and quantification of model compounds (alachlor, diazinon and phenanthrene) using SPME-GC-MS

	Conditions
Autosampler	• 1 min. pre incubation time (85 °C) • 650 rpm agitator speed (5 s) • 5 min. extraction time • 5 min. desorption time
Oven	• 100 °C initial temp. (1 min) • 325 °C maximal temperature
Back inlet	• 270 °C initial temp. • 0.67 bar pressure • 53.2 mL min^{-1} total flow, and helium as carrying gas
Column	• 1 mL min^{-1} flow • 72.5 kPa pressure • 37 cm s^{-1} velocity
MS detector	• 4 min. solvent delay • 1329.4 resulting EM voltage • 150 °C maximum 200 °C MS Quad • 230 °C maximum 250 °C MS source

3.6.2.4 Limit of detection and limit of quantification

In order to detect and quantified all hydrophobic model compounds in fractionation water samples and in ASE extracts of soil and SOM fractions in "distribution experiment" (Chapter 3.7.1), the different chromatographic methods were used. SPME-GC-MS method was used for the detection of hydrophobic substances direct in fractionation water samples without pre-filtering of the sample. The limit of detection (LOD) was calculated from calibration curve according to formula LOD = 3.3 (SD/S), where SD is the standard deviation of the response based upon the residual standard deviation of the regression line and S is the slope of the calibration curve. Limit of quantification was calculated approximating the LOD according to the formula LOQ = 10 (SD/S) (Shabir, 2003). The detection and quantification limits, used for the determination of model compounds in distribution experiment (Chapter 3.7.1), were presented in Table 7.

Table 7. Detection and quantification limits of the different analytical systems used for quantification of model compounds

Compound	Limit of detection (μg L^{-1})	Limit of quantification (μg L^{-1})
	Water samples	
Atrazine (SPME-GC-MS)	0.1499	0.4542
Terbuthylazine (SPME-GC-MS)	0.0854	0.2588
Alachlor (SPME-GC-MS)	0.0913	0.2767
Diazinon (SPME-GC-MS)	0.0345	0.1045
Phenanthrene (SPME-GC-MS)	0.0180	0.0545
	ASE extracts	
Alachlor (GC-ECD)	1.338	4.055
Phenanthrene (GC-FID)	1.396	4.232
Diazinon (GC-FID)	1.729	5.238
Terbuthylazine (HPLC-DAD)	2.991	9.066
Atrazine (HPLC-DAD)	4.901	14.851

3.7 Experimental series

In both experimental series the samples from 3-30 cm soil layer were used.

3.7.1 Spiking of model compounds into soil

3.7.1.1 Recovery test

The spiking procedure was conducted based on the observations made by Northcott and Jones (2001). The 1 g of soil sample was weighed into 5 mL glass vial (graduated V-Vial, Wheaton, USA). The samples of studied soils (fresh and wastewater irrigated) were spiked with 0.1 mL of an acetonitrile stock solution containing 1) atrazine and terbuthylazine, or of 2) alachlor, diazinon, and phenanthrene, respectively (Chapter 3.5). The initial concentration of each model compound in soil was at 10 mg kg^{-1}. After spiking, the acetonitrile was allowed to volatilize for 3 hours. Afterwards vials were loosely covered with aluminium foil. One half of samples was kept at the room temperature (+20 °C) and the other one in refrigerator at (-19 °C) for three months. Treatments included: two studied soils, two stock solutions and two temperatures. After three months, the soil samples containing atrazine and terbuthylazine were extracted using a dichloromethan-acetone mixture, and these containing alachlor, diazinon and phenanthrene were extracted using n-hexan-acetone mixture (the ASE conditions are given in Table 4).

The recovery (μg g^{-1}) of model compounds was calculated as the mean of model compound content in extract (μg g^{-1}) multiply by extract dilution and divided by weight of extracted soil sample and the soil dry matter (Appendix 3).

3.7.1.2 Distribution of model compounds in soil fractions

The 20 mg of soil sample was weighed in an aluminium polished rim and spiked with 2 mL of one of the two stock solutions (Chapter 3.5). The initial concentration of each model compound in soil was at 10 mg kg^{-1} (200 μg). The spiking procedure was carried out as described in Chapter 3.7.1.1. Treatments included: two studied soils, two stock solutions and two temperatures. After three months samples were fractionated as described in Chapter 3.2.2. The water samples from fifteen cycles in which clay fractions were isolated using ultrasonic energy (each procedure contain 1 minute ultrasonic dispersion) were collected, and the content of model compounds was determined by SPME-GC-MS (Agilent, USA). A 5 mL clear aliquot from each sample was carefully removed by a micro-pipette and transferred in a SPME vial filled with 1.5 g of NaCl for improvement of model compounds enrichment in gas phase of SPME cell. The extraction and chromatographic conditions were described in Chapters 3.6.1 and 3.6.2.

3.7.2 Sorption experiments

The soils (100-250 mg), particle-size fractions (10-75 mg of < 1 µm, and 5-50 mg of 1-2 µm), soil mineral matrix (200 mg; Chapter 3.3.2) and clay mineral matrix (50 mg; Chapter 3.3.2) samples were weighed in 10 mL SPME vials, mixed with 0.01 mol L^{-1} $CaCl_2$ solution (used to stabilise the ion strength), and spiked with aliquots from a concentrated methanol stock solution of phenanthrene (10 mg L^{-1} or 100 mg L^{-1}; 99.5 %, Dr Ehrenstorfer GmbH, Augsburg, Germany) to the finally sample volume of 5 mL. The methanol concentration in total solution volume was always less than 1 % to avoid co-solvent effect (Raber and Kögel-Knaber, 1997). For the optimization of solid-to solution ratio and determination of sorption time, the initial concentrations of phenanthrene solution were of 50 µg L^{-1} and of 200 µg L^{-1}, respectively. For determination of sorption isotherms, phenanthrene solutions of varying initial concentrations ranging from 20 and 400 mg L^{-1} for bulk soil, from 20 to 300 mg L^{-1} for < 1 µm fraction, and from 50 to 400 mg L^{-1} for 1-2 µm fraction were prepared. The uptake of phenanthrene on soils and SOM fractions was set from 20 % to 80 % of the initial concentration. Three replicates of each starting concentration were assembled. For each concentration four blank samples without solid phase were prepared (the sorption conditions were the same). The vials were sealed with teflon lined screw caps and horizontally agitated for time ranging from 15 minutes to 7 days on the table shaker at 27 °C (compact shaker KS 15 A, Edmund Bühler, Hechingen, Germany). After that, they were left to settle for one hour. The 1.5 mL of the clear aliquot from each sample was carefully removed with pipette and transferred into a SPME vial filled with 0.45 g of NaCl. The content of phenanthrene in the samples was measured using SPME-GC-MS system. Sorption of phenanthrene was calculated by comparing the analytes' SPME response from two solutions: sample with sorbate and blank sample.

3.7.3 Disposal of wastes

The chemicals rests were deposited in containers for “chlorine containing wastes”, “ethanol containing wastes”, “bromoform containing wastes” and “acetone containing wastes”. The contaminated soil samples and throwaway things such as latex gloves, contaminated glass and contaminated plastic were sorted and deposited in the prepared containers. Glass was soaked in water with Mucasol (Lab Logistics Group GmbH, Meckenheim, Germany) and washed two times in dishwasher, first time with detergent and second time in distillate water.

3.8 Statistical analysis

Statistical analyses were performed using software package Statistica 6.0 (Stat Soft, Inc., Tulsa, USA). The test for significant differences between means was performed using ANOVA procedure and a multiple comparison test (Tukey test, $p < 0.05$) (Kalbitz, 2001; Sá et al., 2001). Statistical significances in correlations between parameters were computed at $p \leq 0.05$, $p \leq 0.01$ and $p \leq 0.001$ and are represented by *, ** and ***, respectively.

4 Results and discussion

4.1 Elemental composition

Elemental analyses were conducted to study the influence of irrigation with treated wastewater, on carbon and nitrogen content in soil and SOM fractions, and on decomposition degree of SOM indicated as C/N ratio. The carbon concentration and its distribution among particle-size and density fractions were the base for the experimental part of the work considering the sorption capacities of soils and SOM fractions.

4.1.1 Bulk soils

The results of elemental composition of bulk soils are presented in Table 1 (Chapter 3.1.2). The total organic carbon (TOC) and total nitrogen (TN) contents in the top soil layer (0-3 cm) were about ten times higher than these in deeper soil layers due to dominance of plant residues. Hence, the soil layers of 0-3 cm were not included in statistical analyses. In the 3-30 cm soil layer of freshwater irrigated soil, higher TOC and TN contents than in treated wastewater irrigated soil were detected. The long-term irrigation with treated wastewater significantly increased TOC and TN contents at 30-120 cm soil depth.

The enrichment of carbon in soils amended with animal manure, sewage sludge and wastewater was already indicated (Gerzabek et al., 2001a; Christensen, 1988). In treated wastewater irrigated soils from Bazra site enrichment of carbon at 30-120 cm soil depth was also found. The influence of treated wastewater irrigation in the deeper soil layers could be related to the effect of translocation of the labile part of SOM such as polysaccharides down in soil profile (Schmidt et al., 2000).

The soils at Bazra site have a very low C/N ratio, which is characteristic for sandy soils with low organic carbon content. The C/N ratio, which varied between 5 and 10, was significantly higher at 30-120 cm soil depth of treated wastewater irrigated soil in comparison with that of freshwater irrigated soil. The published data have shown that organic matter originated from wastewater can be partly (~ 20 %) biodegraded in the root zone (Fine et al., 2002). Moreover, the activity of enzymes, which are responsible for the transformation of plant and animal material in soil, was greater in the root zone between 30 cm and 40 cm of soil depth, and decreased with increasing soil depth

(Niemi et al., 2005). Therefore, the higher C/N ratio in the deeper soil layers of treated wastewater irrigated soil might be resulted from an input of organic matter, which is allocated from the upper soil layers. Although in the deeper soil layers of treated wastewater irrigated soil the TN concentrations were higher than in freshwater irrigated soil, it did not influence the C/N ratios due to the high carbon concentrations.

4.1.2 Hot water extractable fraction

The properties of hot water extracts isolated from Bazra soils are presented in Table 8.

Table 8. Characterization of the hot water extractable fraction isolated from soils of a citrus orchard at experimental site Bazra (Israel); FW - freshwater irrigated soil, WW - treated wastewater irrigated soil; the standard deviation (n = 3) is given in parenthesis

	Elemental content (mg kg^{-1})			
	HWC		**HWN**	
Soil depth (cm)	**FW**	**WW**	**FW**	**WW**
0-3	2469 (58) d[1]	2147 (109) c	384 (11) e	333 (10) d
3-30	367 (29) b	299 (4) b	52 (4) c	33 (0) b
30-60	155 (20) a	161 (9) a	13.8 (2.4) a	12.5 (0.8) a
60-90	98 (20) a	129 (3) a	5.6 (0.2) a	10.3 (1.0) a
90-120	88 (2) a	113 (3) a	4.7 (0.1) a	7.6 (0.5) a

	Contribution to the total amount (%)			
	Contribution of HWC to TOC		**Contribution of HWN to TN**	
Soil depth (cm)	FW	WW	FW	WW
0-3	5.0	3.9	7.7	5.9
3-30	5.0	4.5	6.8	4.8
30-60	5.4	3.1	4.8	2.6
60-90	5.2	2.8	1.4	2.1
90-120	6.8	2.9	1.9	1.7

[1] (a-e) Different letters (a-e) indicate significant difference within a line (between two treatments) and within a column at the $p < 0.05$ probability level (ANOVA analysis, Chapter 3.8)

The values of hot water extractable carbon (HWC) were highly correlated with total organic carbon content (TOC) (freshwater irrigated soil: $y = 20.3x - 0.2$; $r^2 = 0.99$***; treated wastewater irrigated soil: $y = 25.7x + 0.55$; $r^2 = 0.99$***; for each soil n = 15). The relationship between these parameters had been previously reported by Schulz and Körschens (1998). A strong correlation for the nitrogen content of HWF and TN was also obtained (freshwater irrigated soil: $y = 12.4x + 0.19$; $r^2 = 0.99$***; treated wastewater irrigated soil: $y = 16.2x + 0.3$; $r^2 = 0.98$***; for each soil n = 15). Schulz (2004) and Leinweber et al. (1995) found that different fertilisation methods can influence the composition of HWF and that fertilisation effect can be first reflected in the HWF. From these observations and the close correlation between TOC and HWC fraction can be concluded that HWC can be used as a sensible indicator of changes in SOM.

The level of the HWC varied between 2.8 and 6.8 % of the TOC, and the level of hot water extractable nitrogen (HWN) varied between 1.4 and 7.7 % of the TN (Table 8). The contribution of HWF fraction to the TOC in upper soil layers was similar for both freshwater and treated wastewater irrigated soils; however, the contribution of HWF in treated wastewater irrigated soil decreased with increasing soil depth and was two times lower than that of freshwater irrigated soil. The irrigation with treated wastewater decreased the contribution of hot water extractable fraction to the total organic carbon while the concentration of hot water extractable carbon was slightly increased. The decrease in contribution of hot water extractable fraction to TOC was caused by the sorption process of organic matter on soil minerals (Fine et al., 2004; Rumpel et al., 2004).

4.1.3 Clay and specific light fractions

Combined particle-size and density fractionation procedure yielded, mass recoveries ranging from 97 % to 99 % (Figure 4), and TOC recoveries ranging from 79 % to 108 %.

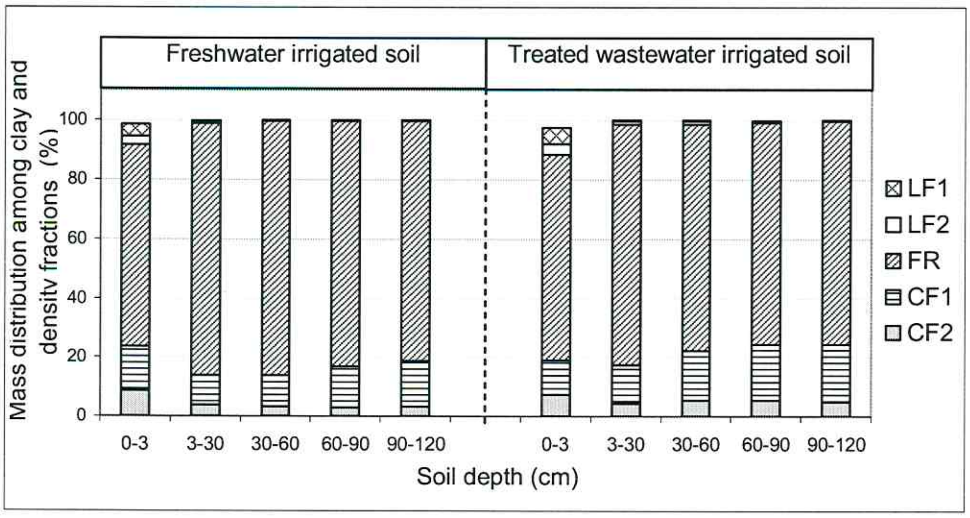


Figure 4. **Percentage mass distribution among clay fractions (CF1: < 1µm; CF2: 1-2 µm), density fractions (LF1: < 1.8 g cm^{-3}; LF2: 1.8-2.0 g cm^{-3}) and fractionation rest (FR); the fractions were isolated from soils of a citrus orchard at experimental site Bazra (Israel) long-time irrigated with freshwater and treated wastewater**

The mass distribution was in accordance with results obtained for soil texture presented in Table 1 (Chapter 3.1.2). At 60-120 cm depth of freshwater irrigated soil the LF1 fraction was depleted. This was accompanied by the decay of silt minerals (Table 1; Chapter 3.1.2). The FR represented particle-size fraction > 2 µm and comprised most of the soil mass (~ 80%). Despite the high contribution of fractionation rest to the soil mass, this fraction contributed only minor part of organic carbon to soil (Table 9). The LF2 fraction made up to 3 % of soil mass and was mainly composed of minerals. The superior part of organic-mineral complexes with density of > 2 g cm^{-3}, defined as a heavy fraction, remained in clay fractions. The similar observations were made for a Gleysol in the work of Leinweber and Schulten (1995).

The carbon and nitrogen concentrations in the SOM fractions are listed in Table 9 and Table 10.

Table 9. Average concentrations (n = 3) of organic carbon in clay fractions (CF1: < 1 µm; CF2: 1-2 µm) and specific light density fractions (LF1: < 1.8 g cm^{-3}; LF2: 1.8-2.0 g cm^{-3}); the fractions were isolated from soils of a citrus orchard at experimental site Bazra (Israel); FW - freshwater irrigated soil, WW - treated wastewater irrigated soil; the standard deviation (n = 3) is given in parenthesis

	TOC in SOM fractions (g kg $^{-1}$)			
	CF1		CF2	
Soil depth (cm)	FW	WW	FW	WW
0-3	123 (1)	141 (2)	200 (1)	239 (6)
3-30	32 (0)	22 (0)	59 (1)	49.4 (0)
30-60	15.1 (0.2)	14.8 (0.6)	30 (1)	34 (1)
60-90	8.2 (0.0)	13.1 (0.3)	15.1 (1.0)	26.3 (0.7)
90-120	5.8 (0.0)	9.7 (0.1)	9.6 (0.2)	22.9 (0.3)
	LF1		LF2	
Soil depth (cm)	FW	WW	FW	WW
0-3	430 (1)	458 (6)	31.0 (1.1)	24.6 (4.7)
3-30	380 (20)	434 (7)	8.0 (0.4)	8.2 (0.8)
30-60	366 (37)	365 (12)	2.3 (0.0)	4.5 (0.4)
60-90	NA[1]	404 (10)	3.0 (0.0)	8.7 (0.3)
90-120	NA	379 (15)	1.6 (0.1)	9.1 (0.1)

[1] NA - not available; no LF1 fraction at 60-120 soil depth was isolated

Independent from the soil depth, the highest carbon concentrations ranging from 360 g kg^{-1} to 460 g kg^{-1} were indicated in LF1 fraction. This was in the same order of magnitude as concentrations of carbon in the < 1.8 g cm^{-3} fraction (325-385 g kg^{-1}) reported by Schulz (2000), and in fractions isolated from silt particle-size fraction (364-407 g kg^{-1}) (Shang and Tiessen, 2000). This fraction seems to be loosely associated to soil minerals or entrapped in aggregates, thus having carbon level independent from mineral composition. Although the fraction LF1 had high carbon concentration, it accounted only for less than 5 % of soil mass.

The CF2 fraction had about two times higher carbon concentration than CF1 fraction. In both clay fractions the carbon concentration was highest in the top soil layer and decreased with increasing soil depth. The carbon derived from treated wastewater was predominately contributed to the top soil layer and indicated as significantly increase in carbon concentrations of clay and density fractions. Moreover, the LF1 fraction was also affected in 3-30 cm soil layer, which suggested the possible lability of the organic matter contained in this fraction.

The contribution of the fractions isolated from the top soil layer to the TOC is presented separately from that of the deeper soil layers due to incomparable high values caused by high content of plant residues (Figure 5).

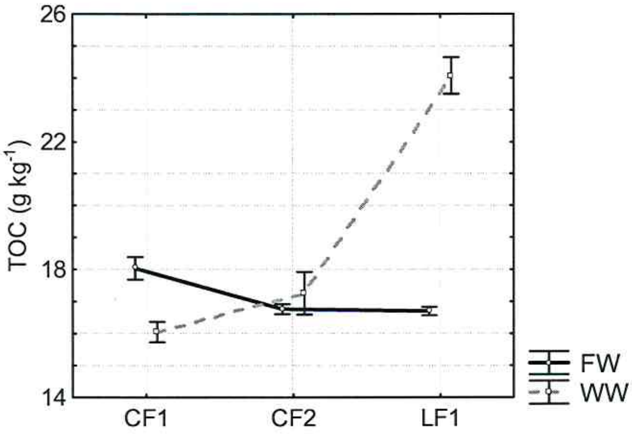


Figure 5. The contribution of organic carbon of clay (CF1: < 1 µm and CF2: 1-2 µm) and specific light density fraction (LF1: < 1.8 g cm^{-3}) to the total organic carbon content (TOC) in the top soil layer (0-3 cm); the fractions were isolated from soils of a citrus orchard at experimental site Bazra (Israel); FW - freshwater irrigated soil, WW - treated wastewater irrigated soil

In Figure 5 it can be demonstrated that the contribution of clay fractions to TOC was: 1) significantly higher for CF1 fraction in freshwater irrigated soil; 2) comparable for

CF2 fraction in both soils. The treated wastewater irrigation enhanced contribution of the LF1 fraction to TOC by 7 % comparing to freshwater irrigated soil. These results evidenced that the carbon pool in the light density fraction is very sensitive to soil management (Alvarez et al., 1998; Alvarez and Alvarez, 2000).

The carbon contribution of the SOM fractions to TOC ($g\ kg^{-1}$) at 3-120 cm soil depth was presented together with the carbon content in bulk soils (Figure 6).

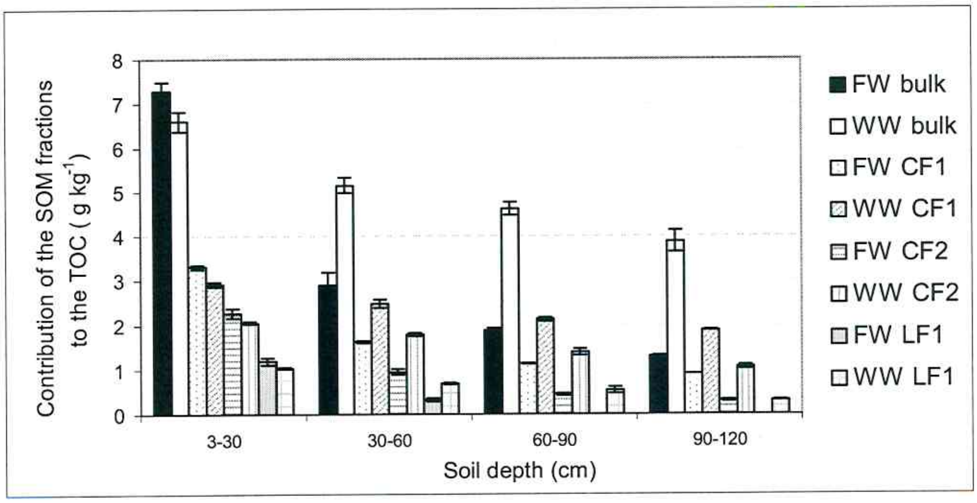


Figure 6. The contribution of organic carbon of clay (CF1: < 1 µm and CF2: 1-2 µm) and specific light density fraction (LF1: < 1.8 $g\ cm^{-3}$) to the total carbon content (TOC) at different soil depths; the fractions were isolated from soils of a citrus orchard at experimental site Bazra (Israel); FW - freshwater irrigated soil, WW - treated wastewater irrigated soil

Clay and light density fractions (in treated wastewater irrigated soil) comprised almost whole carbon and nitrogen at 3-120 cm soil depth. The percentage contribution of clay fractions to the TOC increased with increasing soil depth. Although lower concentration of carbon indicated in the CF1 fraction, the contribution of this fraction to TOC was about twice as high as that of CF2 fraction due to its greater mass in soil. The freshwater irrigated fractions contributed more carbon in the top soil layer whereas in the deeper soil layers treated wastewater irrigated fractions contributed significantly more carbon to SOM. This was due to the greater concentrations of carbon in fractions irrigated with treated wastewater. The highest contribution (40 %) of LF1 fraction to the TOC was indicated in the top soil layer. With the increasing soil depth the contribution of LF1 fraction to the TOC decreased. The LF1 fraction in Bazra soil comprised organic matter loosely associated to minerals. The contribution of this fraction to the decomposable part of SOM could be explained by its presence at 60-120 cm soil depth of treated wastewater irrigated soil.

Table 10. The average concentrations (n = 3) of total nitrogen (TN) in clay fractions (CF1: < 1 µm; CF2: 1-2 µm) and a specific light density fraction (LF1: < 1.8 g cm^{-3}) as well as the contribution of clay fractions to TN in soil; the fractions were isolated from soils of a citrus orchard at experimental site Bazra (Israel); FW - freshwater irrigated soil, WW - treated wastewater irrigated soil; for all results in the table standard deviations was below 5 %.

	TN (g kg^{-1})					
	CF1		**CF2**		**LF1**	
Soil depth (cm)	FW	WW	FW	WW	FW	WW
0-3	14.20	15.40	19.40	21.0	36.2	37.7
3-30	4.20	2.91	6.22	4.83	27.2	30.2
30-60	2.05	1.97	2.72	2.81	16.7	18.8
60-90	1.30	1.51	1.42	2.26	NA[1]	21.7
90-120	0.98	1.40	1.00	2.02	NA	23.8
	TN of bulk soils g kg^{-1}		**Contribution of clay fractions to TN of bulk soils (g kg^{-1})**			
			CF1		**CF2**	
Soil depth (cm)	FW	WW	FW	WW	FW	WW
0-3	4.97	5.62	2.11	1.79	1.67	1.55
3-30	0.77	0.67	0.42	0.39	0.24	0.20
30-60	0.29	0.48	0.22	0.33	0.09	0.15
60-90	0.38	0.5	0.18	0.29	0.04	0.12
90-120	0.25	0.45	0.15	0.28	0.03	0.11

[1] NA - not available; no LF1 fraction at 60-120 soil depth was isolated

The nitrogen concentrations in SOM fractions showed similar trends to carbon concentrations except the LF1 fraction in treated wastewater irrigated soil, which nitrogen content increased at 30-120 cm soil depth. The contribution of CF1 fraction to the total organic nitrogen in soil was higher than that of CF2 fraction (Table 10.). The higher mass of CF1 fraction in soil was decisive.

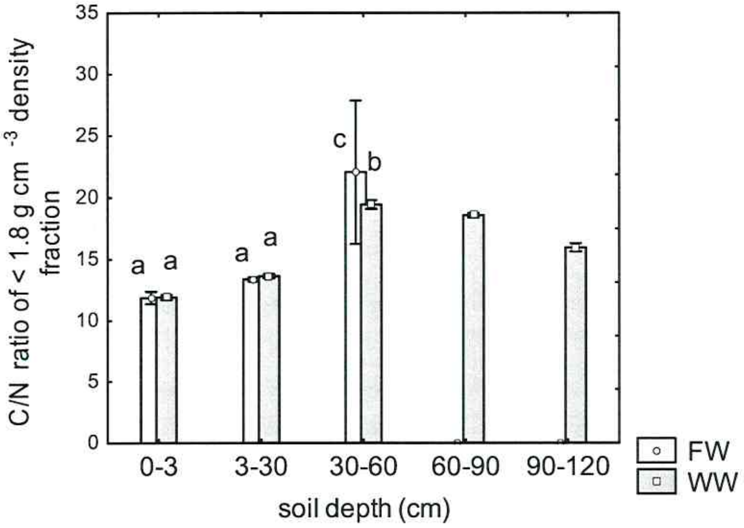


Figure 7. The C/N ratio in a specific light density fraction (LF1: < 1.8 g cm^{-3}) isolated from soils of a citrus orchard at experimental site Bazra (Israel); FW - freshwater irrigated soil, WW - treated wastewater irrigated soil; (a-c) indicate significant difference ($p < 0.05$) between treatments and soil depths (ANOVA analysis, Chapter 3.8)

The C/N ratio serves as an indicator, for the decomposition of SOM fractions, and for the accumulation of recalcitrant material in SOM (Chefetz et al., 1996; Chen and Chiu, 2003). The C/N ratio of LF1 fraction was greater than that of clay fractions, and was increased with increasing soil depth in the root zone (0-60 cm) and slightly decreased at 60-120 cm soil depth (Figure 7). Similar values of C/N to that measured in Bazra soils (11-22) were reported by Six et al. (2001) (about 14) and Golchin et al. (1997) (for < 1.6 g cm^{-3} fraction: C/N = 17-18). The C/N ratio in LF1 fraction was influenced by N content rather than by the C content. The N concentration in LF1 fraction of the upper soil layers was dependent on root material; thus, the C/N ratio increased with soil depth. At deeper soil layers, the content of SOM fractions was strongly dominated by the soil matrix composition (Table 1, Chapter 3.1.2). The silt and clay mineral particles were stored in the deeper soil layers of treated wastewater irrigated soil. The nitrogen compounds in particularly amides contained in SOM may be protected against microbial decomposition and mineralization by physical encapsulation in the mineral dimensional structures (Ding et al., 2002).

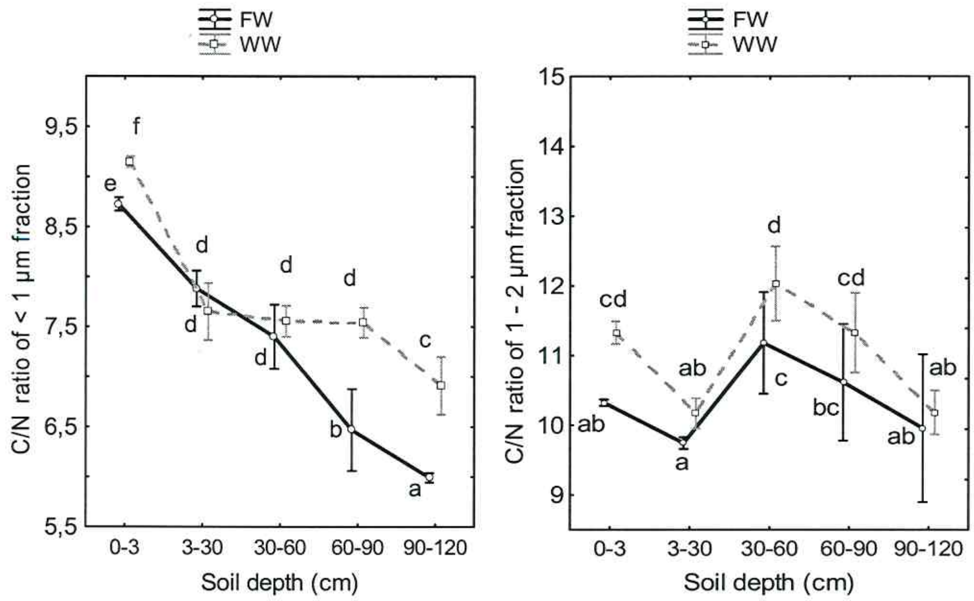


Figure 8. The C/N ratio of clay size fractions (CF1: < 1 µm; CF2: 1-2 µm) isolated from soils of a citrus orchard at experimental site Bazra (Israel); FW - freshwater irrigated soil, WW - treated wastewater irrigated soil; different letters (a-f) indicated significant difference between treatments and soil depth at the $p < 0.05$ probability level (ANOVA analysis, Chapter 3.8)

An increase in nitrogen concentration as well as a decrease in the C/N ratio with decreasing particle size was frequently reported (Gerzabek et al., 2001b; Chen and Chiu, 2003). It was caused by, (1) rapid transfer of nitrogen rich components to the fine particles, (2) relative increase in nitrogen content during decomposition, and (3) higher stabilization of nitrogen-rich compounds (Gerzabek et al., 2001b). The CF2 fraction contained the low decomposed organic matter (high C/N ratio); thus, the C/N ratio was stronger affected by the nitrogen contributed by plant residues (Figure 8). Therefore, the trend of C/N ratio was similar for both freshwater and treated wastewater irrigated fractions. The C/N ratio of CF1 fraction was mostly affected by carbon content and in the minor part by plant residues indicated by constant decrease in C/N with soil depth. The CF1 fraction responded to the application of treated wastewater by a decrease in decomposition degree of organic matter caused by an enrichment of carbon originated from treated wastewater. The wastewater originated organic matter can be accumulated in LF1 fraction and due to the loosely association to soil minerals allocated to the deeper soil layers and stabilized on clay minerals.

4.2 Spectroscopic properties

4.2.1 Bulk soils

The SOM-free spectra of the mineral matrix (Chapter 3.3.1) were substracted from the bulk soil sample spectra and examples are presented in Figure 9. Generally, in the bulk soil spectra the following bands were indicated: at ~ 3360 cm^{-1} (H and OH group bonds), at 2920 cm^{-1} and at 2850 cm^{-1} (aliphatic structures), at ~ 1710 cm^{-1} (COOH, C=O), at 1670-1650 cm^{-1} (amid I, II), at 1640 cm^{-1} (COO^-, C=O, aromatic ring, but also H-O-H stretch bending), at 1560 cm^{-1} (amid I, II), at 1510 cm^{-1} (aromatic rings), at 1446 cm^{-1} and at 1420 cm^{-1} ($-CH_3$, $-CH_2-$ asymmetric bending), at 1400-1370 cm^{-1} characteristic for (COO^-) group and carbonate anions, and at 1100 cm^{-1} and at 1030 cm^{-1} (C-O bands of polysaccharides) (Ding et al., 2002; Celi et al., 1997; Chefetz et al., 1996).

The content of organic matter in Bazra soil was very low; therefore, the differences between treatments were substantial only in the top soil layer. The freshwater irrigated soil from the top soil layer exhibited a pronounced peak at 1555 cm^{-1} (Figure 9), whereas the treated wastewater irrigated soil exhibited significantly higher intensities of aliphatic structures at 1440 cm^{-1}, and of polysaccharides (lignin) in region between 1100 cm^{-1} and 1030 cm^{-1}. Smernik et al. (2003) reported that sewage sludge can contain alkyls with the high molecular mobility. The application of animal manure to the soil was frequently reflected as an enrichment of lignin blocks in soil (Schulten and Leinweber, 1991). The deeper soil layers of treated wastewater irrigated soil were enriched in the protonated carboxylic groups represented by the band at 1710 cm^{-1} and aromatic rings at 1510 cm^{-1} typical for oxidation products.

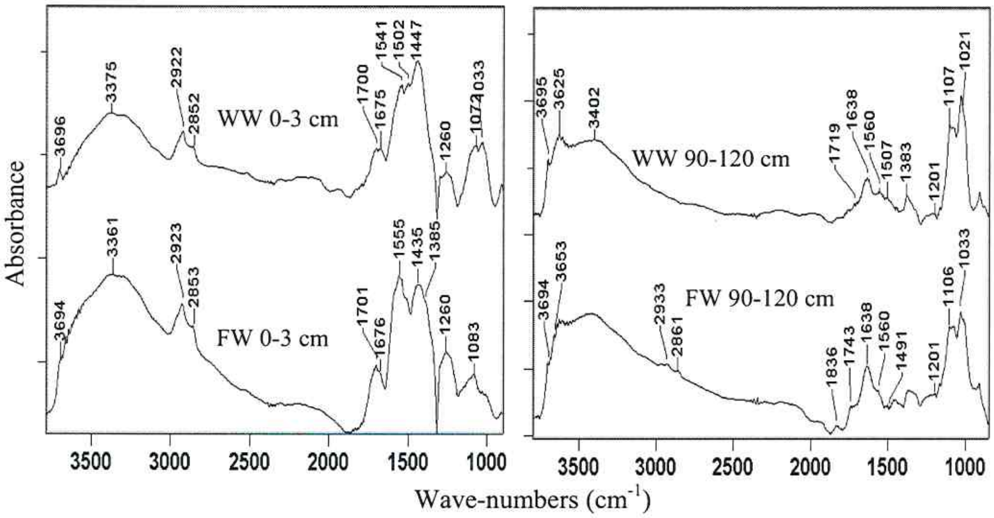


Figure 9. **DRIFT spectra of bulk soils from 0-3 and 90-120 cm soil layers after subtraction of spectra of organic matter-free mineral component; soil samples were taken from a citrus orchard at experimental site Bazra (Israel); FW - freshwater irrigated soil, WW - treated wastewater irrigated soil**

4.2.2 Hot water extractable fraction

Hot water extractable fraction (HWF) was characterized by different spectroscopic methods. Spectroscopic parameters were used to monitor changes in molecular weight (E4/E6), aromaticity (A_{280} - specific absorption coefficient), and polycondensation degree (HIX) of organic matter in hot water extracts caused by treated wastewater irrigation. The E4/E6 ratio, HIX and A_{280} were commonly used in studies of dissolved humic substances and dissolved organic matter (DOM) (Senesi et al., 2003; Grøn et al., 1996; Guo and Chorover, 2003; Kalbitz et al., 2003b). In the present study the values of HIX (5-18) and A_{280} [7-24 L (g C)$^{-1}$ cm^{-1}] of HWF (Table 11) were in the range of these reported for intermediate biodegraded DOM indicated for HIX in the range between 12 and 20, and for A_{280} in the range between 23 and 37 L (g C)$^{-1}$ cm^{-1} (Kalbitz et al., 2003b). The similarities in spectroscopic characteristics of HWF fraction extracted from soil samples collected from 3 to 120 cm soil depth and DOM, suggested comparable proportions of both aliphatic and aromatic constituents as well as similar polycondensation degree of organic matter. The HIX and A_{280} of hot water extracts were positively correlated ($r^2 = 0.72^{***}$, n = 30); moreover, both parameters presented similar trends for freshwater and treated wastewater irrigated soils indicated as an abrupt increase at 3-30 cm soil depth, followed by a decrease at soil depth from 60 to 120 cm. The treated wastewater irrigation significantly decreased HIX and A_{280} in hot water extracts from 90 to 120 cm soil depth and in case of HIX also in extracts from 60-90 soil depth. The E4/E6 ratio measured by UV-VIS spectroscopy was proportional to molecular weight, and inversely proportional to degree of polycondensation of dissolved organic matter. The values of E4/E6 ratio in extracts isolated from different soil layers between 30 and 120 cm depth of freshwater irrigated soil were comparable, whereas in extracts of the treated wastewater irrigated soil, they were significantly increased from 3 to 60 cm soil depth. This was in agreement with significantly decreased values of A_{280} indicated in extracts of wastewater irrigated soil from 3-30 cm soil layer. In contrast to significantly higher carbon and nitrogen contents in SOM fractions and hot water extracts of treated wastewater irrigated soil, neither HIX, nor A_{280}, nor E4/E6 were influenced by the irrigation water quality in the top soil layer. The great part of hot water extractable carbon in the top soil layer was derived from plant organic matter, the main contributor to HWC, which overlapped the effect of organic matter supplied by wastewater.

Table 11. **Spectroscopic parameters characterizing the hot water extractable fraction isolated from soils of a citrus orchard at experimental site Bazra (Israel); average of $n = 3$; FW - freshwater irrigated soil, WW - treated wastewater irrigated soil; (a-f) - different letters indicate significant difference within a line (between two treatments) and within a column at the $p < 0.05$ probability level (ANOVA analysis, Chapter 3.8)**

	Spectroscopic characterization of hot water extractable fraction (HWF)					
	$A_{280\ nm}$ L (g C)$^{-1}$ cm^{-1}		**E_4/E_6**		**HIX**	
Soil depth (cm)	FW	WW	FW	WW	FW	WW
0-3	7.3 (0.2) a	7.3 (0.5) a	12.6 (0.3) cd	13.8 (0.6) cd	5.6 (0.2) a	5.2 (0.1) a
3-30	24.4 (0.6) f	22.9 (0.4) e	15.2 (0.2) d	36.0 (0.5) f	15.9 (1.8) cd	14.7 (2.8) bc
30-60	20.1 (0.7) d	18.99 (0.5) dc	9.1 (0.7) abc	27.3 (0.6) e	17.7 (1.2) cd	15.4 (1.1) bcd
60-90	19.0 (0.4) dc	17.8 (0.3) c	7.4 (1.8) ab	5.3 (1.0) a	18.6 (0.8) d	14.0 (0.6) bc
90-120	18.2 (0.3) c	15.9 (0.9) b	6.7 (1.4) ab	10.5 (4.8) bcd	17.3 (1.5) cd	11.6 (0.8) b

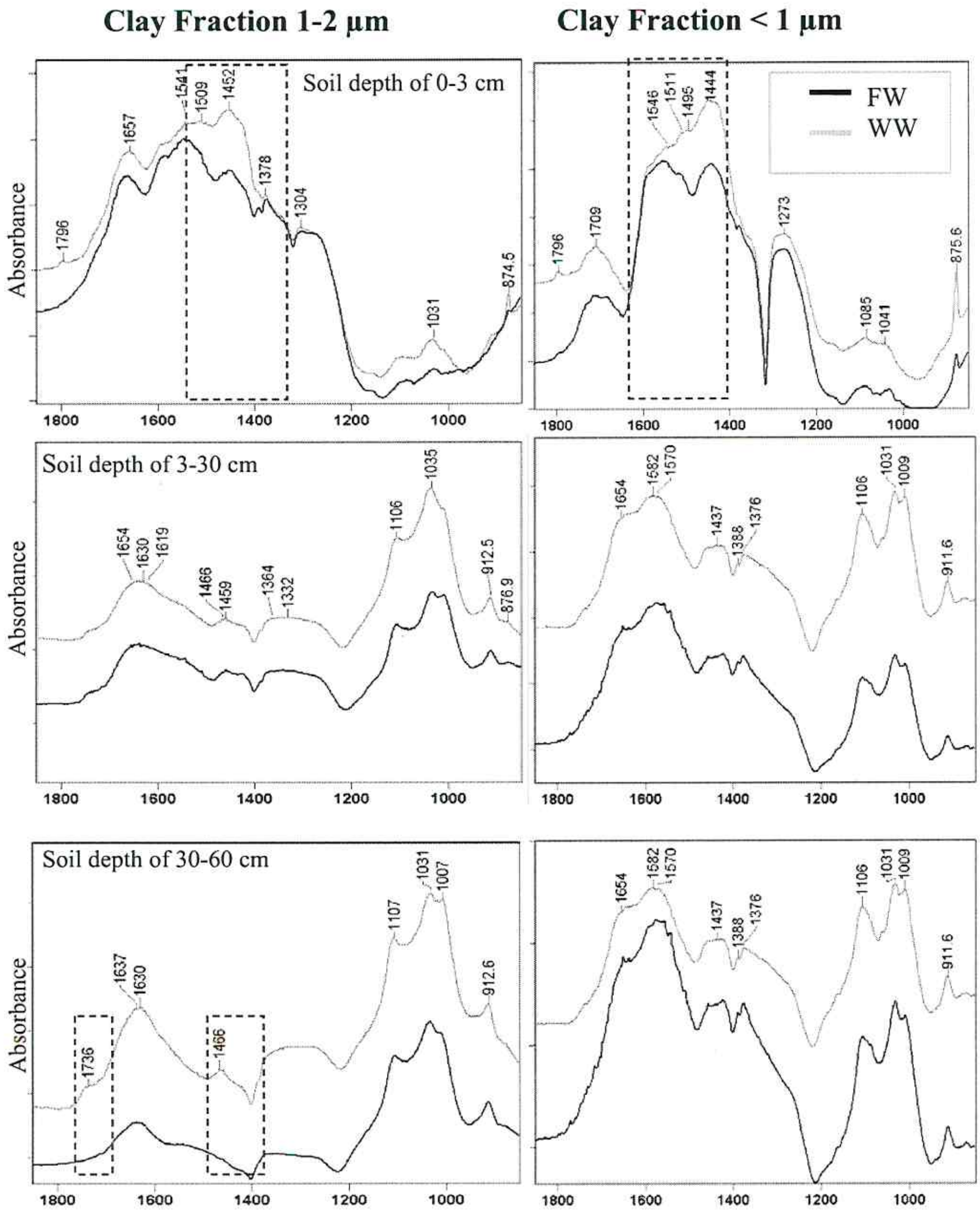
The wastewater irrigation resulted in alteration of organic matter in the hot water extractable fraction, the lower aromaticity in samples from 3-30 and 90-120 cm soil layers as well as the lower decomposition degree of organic matter at soil depths from 60 to 120 cm. The results obtained for A_{280} and HIX were in agreement with these obtained for E4/E6 ratio, indicating a decrease in weight of organic molecules in extracts from 3-30 and 30-60 cm soil layers.

It seems reasonable to resume that changes in the proportions of the aliphatic and aromatic structures in the HWF resulted from the sorption of hydrophobic, aromatic constituents with high molecular weight on the soil minerals and replacement of aliphatic, hydrophilic constituents from mineral surface to the hot water extractable fraction. The wastewater derived organic matter was predominantly accumulated in the hot water extractable fraction forcing the gradually elution of hydrophilic molecules into the deeper soil layers. Several batch studies proposed the sorption model in which the competitive adsorption of effluent derived organic matter containing moieties having high affinity for mineral surface and displacement of weakly bound SOM occurred (Kaiser et al., 1996). The explanation for the alteration of HWF in the deep soil layers can be related to the composition of organic matter in HWF and its week stabilisation on soil minerals. The HWF comprises carbohydrates, amino acids, amides and heterocyclic N-containing compounds as well as acetamide, which is a typical product of bacteria cells (Leinweber et al., 1995). The labile part of HWF in soil can be easily allocated into the deeper parts of soil horizon by the water delivered from the drip irrigation system. The presence of great number of free adsorption sites on the minerals surface in the deeper soil layers caused, by low organic matter content, and high content of clay minerals in soil resulted in the sorption of organic matter rich in carboxylic groups on Fe/Al oxides and reduced contribution of hot water extractable fraction to TOC. These conclusions are based on the recently studies of Gigliotti et al. (1997) and Guo and Chorover (2003). The first publication, reported contribution of municipal waste compost to DOM displayed as an increase in hydrophilic polysaccharides and a decrease in olefin group content. Guo and Chorover (2003) reported that in DOM solution pumped through the soil column, the fractions with the higher molecular weight, higher molecular absorptivity (ε_{280} expressed in L mol^{-1} C cm^{-1}), higher molecular size of DOM molecules (the ratio of UV-VIS absorbances at 250 nm and 365 nm), lower acidicity, and lower hydrophilicity were preferentially adsorbed on soil minerals.

4.2.3 Clay fractions

The DRIFT spectra of clay fractions (< 1 μm and 1-2 μm) isolated from the 0-3 cm, 3-30, 30-60, 60-90 cm soil layers, obtained by subtraction of the spectrum of H_2O_2

treated fraction (Chapter 3.3.1) from the whole fractions spectra are presented in Figure 10. The spectrum of clay fractions from 90-120 cm soil layer did not differ from that of isolated from 60-90 cm soil layer. The main changes in peak intensities were detected in range between 1800 cm^{-1} and 850 cm^{-1}; therefore only this part of the spectra was presented.



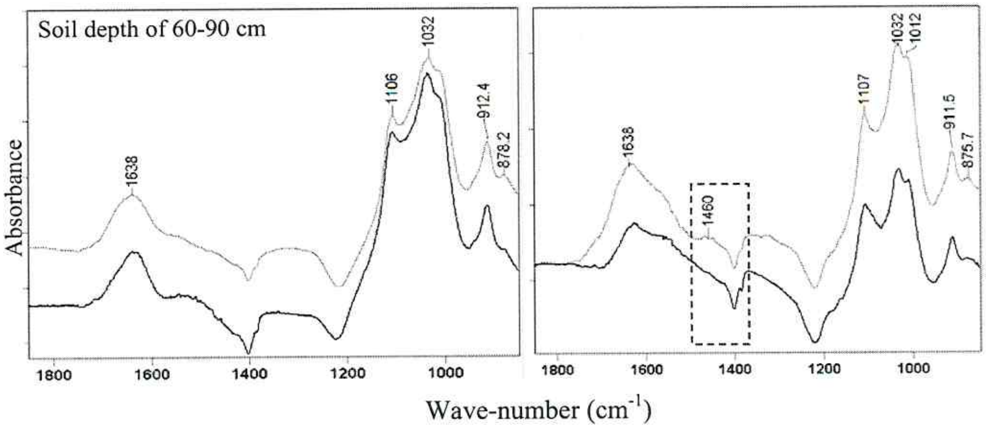


Figure 10. DRIFT spectra of clay fractions (CF1: < 1 µm and CF2: 1-2 µm), isolated from the different soil layers, after subtraction of organic matter free spectra; each spectrum is an average of 3 replications; the fractions were isolated from soils of a citrus orchard at experimental site Bazra (Israel); FW - fraction isolated from freshwater irrigated soil; WW - fraction isolated from treated wastewater irrigated soil

The main bands were indicated: at 1710 cm^{-1} (protonated carboxylic groups), at 1657 cm^{-1} (C=O in amid I and II), at 1590 cm^{-1} (C=C aromatic ring vibrations), at 1545 cm^{-1} (N-H; C=N stretching in amid II), at 1508 cm^{-1} (aromatic stretch C=C), at ~1460 cm^{-1} assigns to (-CH_3, -CH_2- asymmetric bending), at 1380 cm^{-1} (COO^-, OH phenol and water, or aromatic bands), and at 1030 cm^{-1} (polysaccharides) (Ding et al., 2002; Landgraf et al., 1998; Madejová, 2003; Spaccini et al., 2001; Majcher et al., 2000; Haberhauer et al., 1998). At 0-3 cm soil depth, the spectra of CF1 fraction heaving no peak at 1710 cm^{-1}, and intensive peak at 1657 cm^{-1} differed from these of CF2 fraction. In the spectra of CF2 fraction at 3-30 and 30-60 cm soil depths, the amide peak at 1650 cm^{-1} appeared, whereas the composition of organic matter in the CF1 fraction characterized by peaks at 1640 cm^{-1} (deprotonated carboxylic groups, water), at 1460 cm^{-1} (-CH_3, -CH_2- asymmetric bending) and at 1030 cm^{-1} (polysaccharides) became constant. Below the root zone (60-90 and 90-120 cm soil layer) the CF2 fraction contained decompose organic matter indicated as a peak at 1640 cm^{-1} (deprotonated carbocylic groups, water), although, the rest of plant material was still indicated as an amide band at about 1560 cm^{-1}. In subsoil the CF2 fraction was enriched in amides structures originated from plant rest, while the CF1 fraction was dominated by intense peak at 1640 cm^{-1} characteristic for C=O group.

In the top soil layer, the wastewater derived organic matter was contributed to TOC of both fractions, and was indicated by intense bands corresponding to C-H and to C-O bonds of polysaccharides (in CF1 fraction at 1030 cm^{-1}), $-CH_3$, $-CH_2-$ asymmetric vibration (1450 cm^{-1}), amide II or aromatic ring vibration (1510 cm^{-1}), and amid group vibration (1540 cm^{-1}). Several studies indicated similar effects of DOM application to soil (Guo and Chorover, 2003; Gigliotti et al., 1997). Enrichment of amid constituents in the top soil layer may imply an input of nitrogen constituents from treated wastewater. The changes in organic matter of CF2 fraction isolated from 3 to 60 cm soil depth caused by irrigation, were covered by the peaks of amide (1650 cm^{-1}, 1570 cm^{-1}) typical for the plant residues. Unfortunately, in the spectra of subsoil, the band assigned to polysaccharides at 1030 cm^{-1} was difficult to interpretation due to the strong influence of mineral components of fraction in this part of spectrum. The wastewater irrigation caused the appearance of peak of aliphatic structures (1450 cm^{-1}) in spectra of clay fractions at 30-90 cm soil depth. This fact supported findings from previous chapters (Chapter 4.1.2; 4.1.3) considering 1) the alteration of water extractable organic matter in soil irrigated with treated wastewater, 2) the translocation of hydrophilic organic matter from the upper soil layers down in soil profile, 3) adsorption of aliphatic constituents derived from treated wastewater on clay minerals. Therefore in subsoil, the decomposition degree of organic matter isolated from treated wastewater irrigated soil, expressed as C/N ratio, was lower than that of freshwater irrigated soil. In the upper soil layers where the competition between organic molecules for the sorption sites might occur, the treated wastewater derived organic matter was only partly adsorbed on mineral surface due to low degradation degree of organic matter. The hydrophilic compounds in soil solution (for example these of HWF fraction) can be allocated down in soil horizon and stabilised on clay minerals (Schmidt et al., 2000). In the root zone the bacterial activity is high and organic matter can be decomposed (Niemi et al., 2005; Fine et al., 2002), which resulted in the formation of aliphatic and alkyl structures and an increase in carboxylic group content having high affinity for clay surface. The sorption on clay minerals is based on adsorption of organic matter on Fe/Al oxides by ligand exchange of acidic groups for surface hydroxyls groups (Guo and Chorover, 2003). The size of mineral particles influences the kind of interaction between mineral surface and organic matter. The CF2 fraction is able to interact through the electrostatic bonds with large organic molecules with quite unmodified structure (Van der Waals forces) (Madejová, 2003). Therefore in Bazra soils, the organic matter associated to this fraction had lower decomposition degree (C/N = 10-12) and contained plant derived organic matter such as amides. The organic molecules can replace water molecules from mineral surface, resulting in a decrease of hydration energy of organic molecules, and their hydrophobic character (Madejová, 2003). Some studies reported that organic matter delivered to the soil was rapidly trapped in the fine clay particle size fraction (Desjardins et al., 2004; Chen and Chiu, 2003; Schulten and Leinweber, 1991). Thus, the clay fraction CF1 in Bazra soil accumulated organic material rich in carboxylic groups (1640 cm^{-1}), typical oxidation product contained in recalcitrant organic material.

4.2.4 Specific light density fractions < 1.8 g cm^{-3} (LF1)

The DRIFT spectra of LF1 fraction are presented in Figure 11. They could be quantified due to the high content of organic matter in the fraction. In Table 12, peaks intensities were expressed in K-M intensities (rKM) representing the percentage contribution of organic matter groups to the TOC of the fraction (Solomon et al., 2005; Haberhauer et al., 1998; Haberhauer and Gerzabek, 1999).

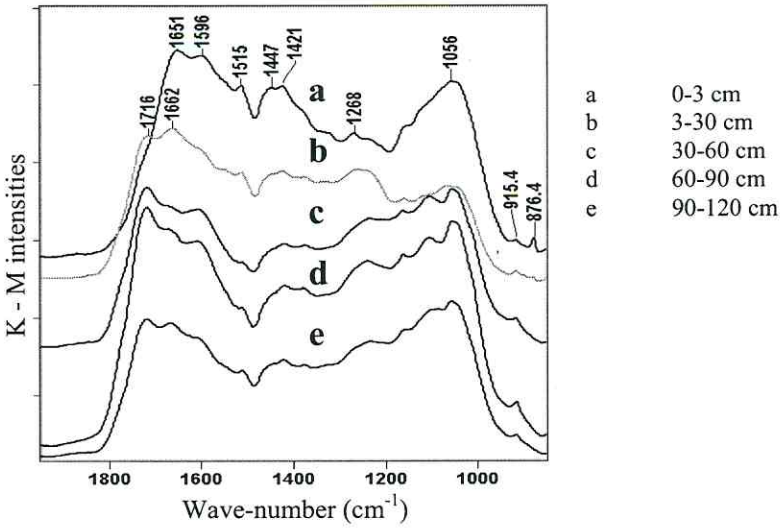


Figure 11. DRIFT spectra of a specific light density fraction (LF1: < 1.8 g cm^{-3}) isolated from different soil layers of soils of a citrus orchard at experimental site Bazra (Israel) irrigated with treated wastewater.

The main bands occurred: at 1720 cm^{-1} (protonated carboxylic groups), small peak at 1660 cm^{-1} (C=O in amid I and II groups), a shoulder at 1610 cm^{-1} (deprotonated carboxylic groups, aromatic rings), a small sharp peak at 1510 cm^{-1} (C=C of aromatic rings, lignin), small band at 1450 cm^{-1} (-CH_3, -CH_2-, asymmetric bending), band at ~1400 cm^{-1} (-CH_3, -CH_2- vibration, C-OH deformation of COOH, COO^- symmetric stretching vibration), broad peak at 1240 cm^{-1} (C-OH asymmetry. vibration, OH deformation of COOH, C-OH vibration), and a peak at 1050 cm^{-1} (C-O stretch of polysaccharide) (Ilani et al., 2005; Celi et al., 1997). The relative K-M intensities at 1420 cm^{-1} and at 1450 cm^{-1} (CH_3- and -CH_2-), at 1510 cm^{-1} (aromatic ring vibrations), at 1670 cm^{-1} (amid I and II groups) and at 2920 cm^{-1} (CH_3- and -CH_2-) decreased with an increasing soil depth up to 30-60 cm soil layer (Table 12). The same intensities showed increasing trend at 60-120 cm soil depth. The inverse trends for the intensities at 1720 cm^{-1} (C=O) and at 1240 cm^{-1} (C-O asymmetric stretch vibration, OH deformation) were indicated. The polysaccharides band at 1030 cm^{-1} was the main contributor to the TOC in LF1 fraction of treated wastewater irrigated soil. At 30-90 cm soil depth, the C=O functional group (1720 cm^{-1}) constituted an average of 20 % of TOC in LF1 fraction isolated from treated wastewater irrigated soil.

Table 12. The average relative Kubelka - Munk (K-M) intensities (%) for the selected wave-numbers (cm^{-1}) calculated from DRIFT spectra (n = 3-4) of a specific light denity fraction (LF1: < 1.8 g cm^{-3}) isolated from freshwater and treated wastewater irrigated soil; no LF1 fraction was isolated from freshwater irrigated soil at 60-120 cm soil depth

	Relative K-M intensities(%)									
Soil depth (cm)	**2920**	**1720**	**1660**	**1600**	**1510**	**1450**	**1420**	**1370**	**1240**	**1050**
	LF1 fraction isolated from freshwater irrigated soil									
0-3	6.8	0.0	7.2	4.1	4.5	7.0	6.3	0.9	1.5	61.7
3-30	4.2	7.6	3.7	2.5	3.3	4.4	3.7	1.0	1.5	68.1
30-60	4.8	13.5	2.2	7.4	2.4	3.8	4.1	1.4	2.8	57.6
	LF1 fraction isolated from treated wastewater irrigated soil									
0-3	7.6	0.0	11.3	11.4	4.2	8.4	7.2	0.4	3.9	45.6
3-30	5.3	10.0	6.0	5.0	3.7	5.5	4.8	1.1	5.0	53.6
30-60	4.3	19.5	1.2	7.6	1.4	4.5	3.8	1.2	6.5	50.0
60-90	4.4	17.1	2.5	6.9	1.8	4.7	3.9	0.9	7.1	50.6
90-120	5.0	12.8	4.4	5.8	3.1	5.5	4.6	0.7	5.5	52.6

In freshwater irrigated soil, the LF1 fraction was present in soil layers, where the input of plant derived organic matter occurred, that means in top soils and root zone (0-60 cm soil depth). In treated wastewater irrigated soil, the root residues and their transformation products also influenced strongly organic matter of LF1 fraction. The absence of protonated carboxylic groups (1720 cm^{-1}) in the fraction from the top soil layer was due to the high value of fresh plant material. The high proportions of polysaccharides in the LF1 fraction can explain its high mobility in soil (Rumpel et al., 2004). With increasing soil depth, the organic matter in LF1 fraction of wastewater irrigated soil was enriched in oxidation products having carboxylic groups (1720 cm^{-1}; 1600 cm^{-1}), and hydroxyl groups (1240 cm^{-1}) which can be preferentially adsorbed on clay minerals at the deeper soil depths (Kaiser et al., 2002; Rumpel et al., 2004). Amides having lower affinity for mineral surface can be eluted down in soil profile. Ding et al. (2002) suggested that the N components may be protected by physical encapsulation in soil minerals in the deeper soil horizons. That can explain the presence of amid structures in the deeper soil layers of treated wastewater irrigated soil. With increasing soil depth, the changes in intensities of aromatic structures (1512 cm^{-1}) were comparable to these of aliphatic bands. In LF1 fraction from the deeper soil layers, the higher content of aromatic structures can be caused by the formation of complexes between hydrophobic molecules and dissolved organic matter and their transport down in soil horizon (Seol and Lee, 2000). An increase in proportion of aliphatic constituents in the deeper soil layers can be caused by decrease in sorption capability of soils irrigated with wastewater and transport of aliphatic compounds down in soil horizon.

In contrast to the clay fractions, the density fractions isolated from the treated wastewater irrigated soil contributed proportionally 15 % less polysaccharides to TOC than that isolated from freshwater irrigated soil in the top soil layer. These results suggested the enrichment of other, than polysaccharides, chemical structures in treated wastewater irrigated fraction, for instance: aliphatics (at 2920 cm^{-1}, 1450 cm^{-1}, 1420 cm^{-1}), amides (at 1660 cm^{-1}) and deprotonated carboxylic groups (at 1600 cm^{-1}).

4.2.5 Conclusions: the influence of treated wastewater irrigation on elemental and spectroscopic parameters of bulk soil and SOM fractions

Summarising, the treated wastewater irrigation influenced the elemental composition, and the spectroscopic characteristic of SOM fractions. As expected, the wastewater derived organic matter supplied to the soil was predominately contributed to the top soil layer, and indicated as significantly higher proportion of carbon and nitrogen in clay fractions, and hot water extractable fraction. In spectra of clay fractions the enrichment of polysaccharides, amides, and aliphatic constituents was indicated. In hot water extractable fraction from the topsoil the irrigation effect was overlapped by the high content of plant derived organic matter.

The most characteristic for treated wastewater irrigated soil from Bazra was the allocation of organic matter down in soil profile. The treated wastewater irrigation altered the composition of organic matter in hot water extractable fraction. During irrigation process the fraction of lower affinity for sorption sites in SOM was replaced by highly hydrophobic structures containing carbonyl groups, and gradually eluted. Therefore, the hot water extractable fraction became more aliphatic and contained organic matter of low decomposition degree. The constituents having lower affinity for clay minerals were transported down in soil profile and partly decomposed in the root zone.

In the treated wastewater irrigated soil, the phases of adsorption of organic matter on clay minerals can be demonstrated by the trend of C/N ratio in CF1 fraction (Figure 6). It was first abruptly decreased at 0-30 cm soil depth and then released constant at the soil depth of 30-90 cm, whereas the trend for freshwater irrigated soil decreased gradually. It means, that adsorption of organic matter originated from treated wastewater on clay minerals decreased the decomposition degree of organic matter in CF1 fraction. In the deeper soil layers, the high clay mineral content, and the low organic matter content enable the sorption of hydrophilic organic molecules having low affinity to mineral surface.

Accounting for changes in LF1 fraction spectra, this fraction can play the role of sink for treated wastewater organic matter. The LF1 fraction was enriched in carboxylic group and aliphatic constituents in the top soil layer. The carbohydrates were the main contributor to TOC of LF1 fraction; thus, this fraction might be eluted down in soil horizon. At 60-120 cm soil depth, the molecules containing carboxylic and hydroxyl groups were adsorbed on clay minerals indicated as decrease in intensities of these groups in DRIFT spectra. The amides, aromatics and aliphatic constituents showed inverse trends to carboxylic and hydroxyl groups. The aliphatic and aromatic constituents represented hydrophobic, and amides represented the hydrophilic fraction of LF1. The changes in DRIFT spectra of LF1 fraction evidenced the transport of hydrophobic molecules down in soil profile. This may be caused by the formation of complexes between hydrophobic molecules and DOM. The treated wastewater derived organic matter could be a source of such DOM in Bazra soil.

4.3 Influence of irrigation water quality on distribution of model compounds in SOM fractions

Understanding of sorption mechanisms of hydrophobic contaminants on SOM fractions is essential for prediction of their degradation time, and potential for leaching to groundwater (Taylor et al., 2004). The aim of this experiment was to investigate the influence of irrigation water quality and the aging temperature on the distribution of model compounds in SOM fractions. The significant influence of the land use on the accumulation of xenobiotics such as terbuthylazine, alachlor and acetochlor was indicated in the upper soil layers (Kalbitz, 2001; Ma et al., 2004; Ismail and Kalithasan, 1997; Taylor et al., 2004). Therefore, the sorption on soils from 3-30 cm soil layer was tested.

4.3.1 Recovery test

The recovery test should provide information about the extractability of model compounds (phenanthrene, alachlor, diazinon, atrazine and terbuthylazine) supplied to soil, and three months aged at different temperatures (Figure 12; Appendix 3). The results will constitute the base for the further calculations of distribution of model compounds among clay and density fractions. Soils irrigated with freshwater and treated wastewater had no extractable phenanthrene, atrazine, terbuthylazine, diazinon and alachlor in background.

The hydrophobicity of model compounds increases in the order: alachlor < atrazine< terbuthylazine < diazinon < phenanthrene (Table 3, Chapter 3.5; Krauss and Wilcke, 2002; Gerstl et al., 1997; Nemeth-Konda et al., 2002; Chefetz et al., 2004). The considerable lower recoveries of the most hydrophobic phenanthrene, and diazinon were caused by their high affinity for SOM. These two compounds can be adsorbed on the SOM decreasing their availability for the extractantas.

Significant lower recoveries of model compounds in soils stored at the room temperature in comparison to that stored in freezer were indicated. Recoveries were decreased by 13 % and 18 % for diazinon and by 20 % and 15 % for phenanthrene, in freshwater and treated wastewater irrigated soil, respectively. For atrazine the significant lower recovery in treated wastewater irrigated soil stored at the room temperature was indicated. Soil freezing limited the diffusion processes; therefore, hydrophobic substances cannot penetrate the structure of humic substances and remain accessible to solvents (Lesan and Bhandari, 2003). The freezing of soil or soil fractions may also increase the extractability of the hydrophobic substances (Krauss and Wilcke, 2002).

The treated wastewater irrigation did not significantly influence the recovery of model compounds.

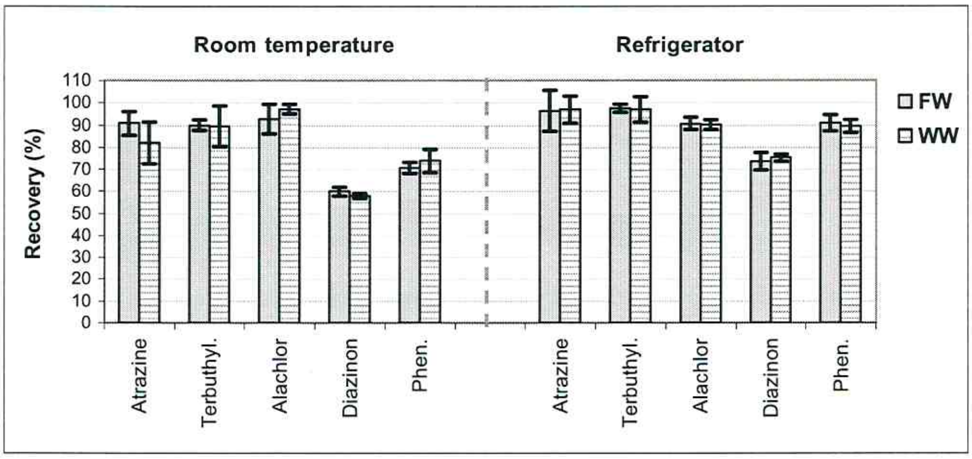


Figure 12. The average recovery (n = 3) of model compounds (atrazine, terbuthyl. - terbuthylazine, alachlor, diazinon, phen. - phenanthrene) in soils of a citrus orchard at experimental site Bazra (Israel); FW - freshwater irrigated soil; WW - treated wastewater irrigated soil; soils after spiking with model compounds were 3 months aged at room temperature (+24 °C) and in refrigerator (-19 °C)

4.3.2 Accumulation of model compounds in clay fractions

The contribution of clay fractions to the total content of model compounds in soil (Figure 13; Appendix 4 and 5) was calculated basing on the bulk soil recoveries. The model compounds were mainly accumulated in clay fractions of Bazra soil due to their high concentration of TOC.

The CF1 fraction accumulated approximately from 1.6 to 3.6 times more atrazine and terbuthylazine, 2.5 times more phenanthrene, and from 3.2 to 4.6 times more diazinon and alachlor than the CF2 fraction. It was, in spite of two times higher carbon concentration in CF2 fraction (Table 9, Chapter 4.1.3). The explanation for these results can be found in mass distribution indicating three times higher content of CF1 fraction compared to CF2 fraction in Bazra soil. The larger differences for alachlor and diazinon can be regarded to the higher affinity of these compounds for mineral surface of CF1 fraction. Both compounds have ether or carboxyl groups which are able to bind by ligand exchange with Fe/Al oxides on clay mineral surface.

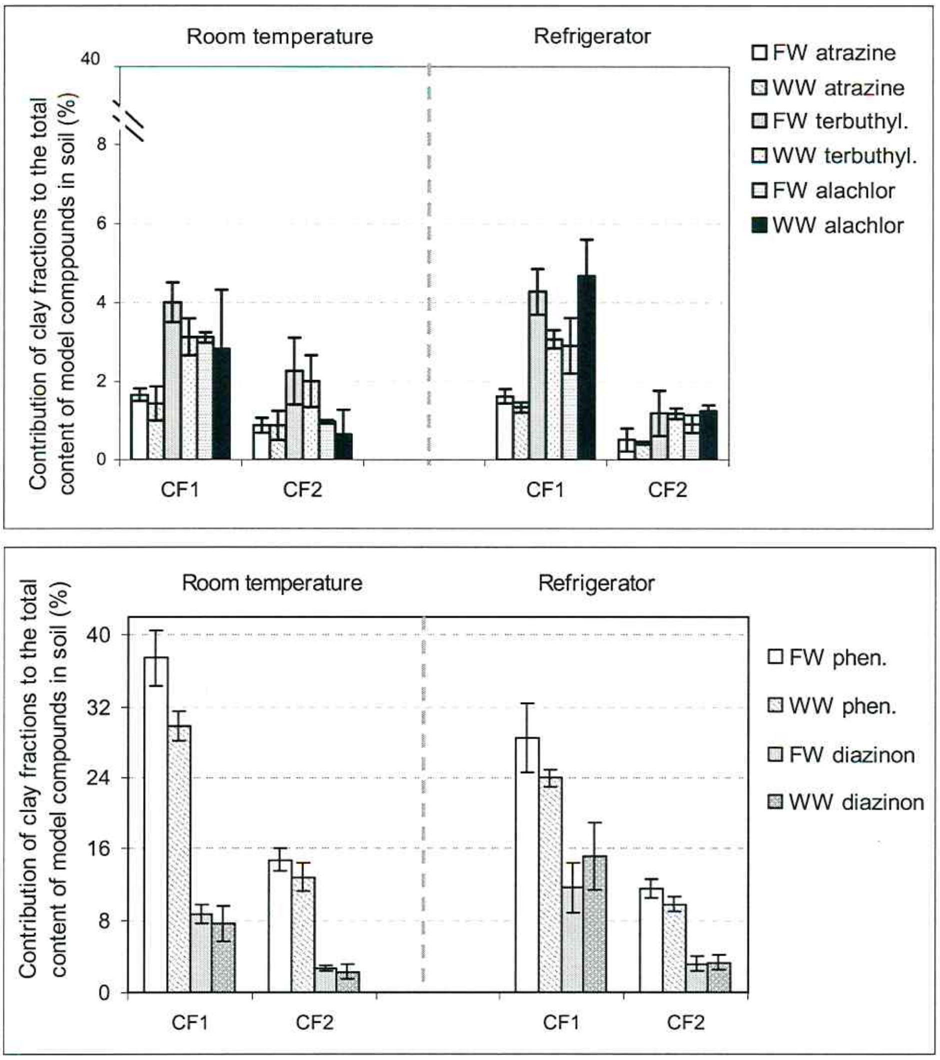


Figure 13. Percentage contribution of clay fractions (CF1: < 1 µm; CF2: 1-2 µm) to the total content of model compounds (atrazine, terbuthyl. - terbuthylazine, alachlor, phen. - phenanthrene, diazinon) in soil (calculation basis: concentration of the model compounds of recovery test; Chapter 4.3.1); the fractions were isolated from soils of a citrus orchard at experimental site Bazra (Israel); FW - freshwater irrigated soil, WW - treated wastewater irrigated soil; soils after spiking with model compounds were 3 months aged at room temperature (+24 °C) and in refrigerator (-19 °C)

The differences between contribution of freshwater and treated wastewater irrigated clay fractions to total content of model compounds (atrazine, terbuthylazine, alachlor and diazinon) in soil were not significant. An exception was indicated for CF1 fraction from freshwater irrigated soil stored at the room temperature, which contributed significantly greater proportion of phenanthrene than the CF1 fraction from treated wastewater irrigated soil.

In soil stored at the room temperature, the contribution of CF1 fraction to total phenanthrene content was significantly higher than the contribution of fraction isolated from soil kept in refrigerator. The same result was indicated for CF2 fraction. Lesan and Bhandari (2003) and Drori at al. (2005) described sorption to the SOM as two stages process, first rapid sorption of hydrophobic compound to the rubbery SOM or to the mineral surface, and the second slow gradually migration of hydrophobic molecules into the micropores and active sites of glassy SOM. This evidenced that the organic matter in clay fraction has glassy structure. The freezing process suppressed the diffusion of model compounds deep in SOM structures.

The adsorption of hydrophobic substances on the SOM fraction was reported as dependent on "quantity" and "quality" of SOM (Zhou et al., 2004). To test this hypothesis, the concentration of model compounds was normalized to organic carbon concentration in clay fractions (μg $(g\ C)^{-1}$) (Figure 14). The results confirmed that irrigation with treated wastewater did not significantly influence the sorption capacity of clay fractions. However, in soils stored at the room temperature, atrazine and terbuthylazine had higher affinity for organic matter in CF1 fraction, and phenanthrene had higher affinity for organic matter in CF2 fraction isolated from freshwater irrigated soil than for organic matter in fractions from treated wastewater irrigated soil.

The organic matter in CF1 fraction was able to adsorb the greater proportions of hydrophobic substances than that in CF2 fraction. The characterization of the clay fractions with DRIFT spectroscopy enable to formulate some thesis considering the chemical composition of these fractions (Figure 10, Chapter 4.2.3). It was indicated that CF2 fraction had a great amount of compounds containing nitrogen, typical for plant litter. The spectra of CF1 fraction showed the presence of hydrophobic aromatic and aliphatic structures which are able to constitute the bindings (hydrogen forces or π - π interaction) with aromatic rings of model compounds (Zhou and Pignatello, 2005).

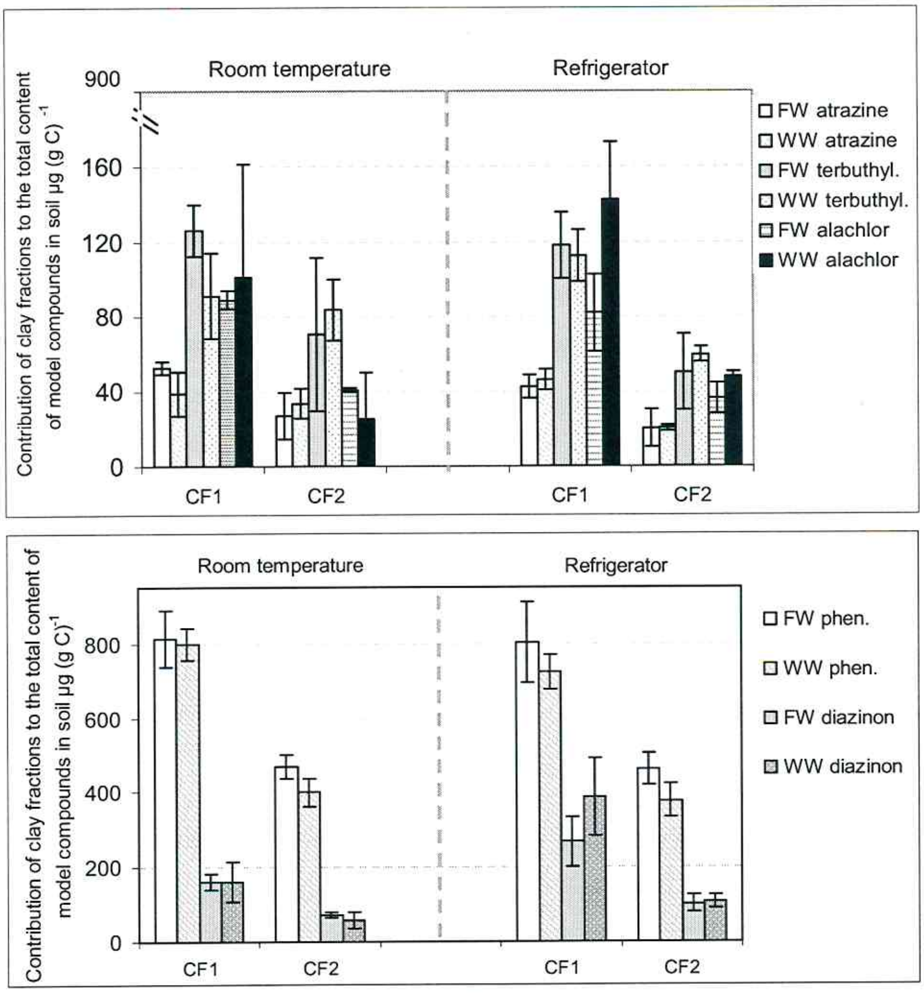


Figure 14. Contribution of clay fractions (CF1: < 1 µm; CF2: 1-2 µm) to the total content of model compounds in soil (atrazine, terbuthyl. - terbuthylazine, alachlor, phen. - phenanthrene, diazinon) normalised to carbon content in the fraction; the fractions were isolated from soils of a citrus orchard at experimental site Bazra (Israel); FW - freshwater irrigated soil, WW - treated wastewater irrigated soil; soils after spiking with model compounds were 3 months aged at room temperature (+24 °C) and in refrigerator (-19 °C)

4.3.3 Accumulation of model compounds in specific light fractions

The specific light density fractions of Bazra soil contributed very little to the total content of hydrophobic substances in soil. The LF2 fraction had minor concentration of carbon. Although the LF1 fraction contained 40 % of carbon, it constituted only 5 % of soil mass. The accumulation of model compounds in LF1 fraction is presented in Figure 15. The standard deviations are very high, due to the small amounts of isolated fraction. The composition of organic matter in LF1 fraction was essential for the sorption processes of hydrophobic substances. The organic matter in this fraction was poor in aromatic ring content (2-5 % of organic matter content) and in hydrophobic aliphatic groups (about 5 %); however, it was rich in polysaccharides (45-68 %) and carboxylic groups (7.6-10 %) detected by DRIFT spectroscopy (Table 12, Chapter 4.2.4). Atrazine and terbuthylazine as triazines seems to have higher affinity for organic matter in LF1 fraction due to the presence of polar amine groups in their molecules. The triazines are able to constitute ionic bonds with carboxylate groups of SOM (Senesi et al., 1995; Martin-Neto et al., 2001). The affinity of organic matter for phenanthrene was frequently attributed to its aromaticity calculated from NMR spectra (Xing, 1997) or its polarity calculated from the amount of C domains containing N, O, or S atoms (Huang and Lee, 2001). Only small amounts of diazinon and no phenathrene were detected in specific light density fraction. Phenathrene can form π - π electron donor-acceptor interaction with π acceptor sites in SOM, but it is not able to bind complexes with hydroxyl, phenolic and carboxylic groups. The results obtained for phenanthrene and diazinon but also for the other model compounds confirmed low affinity of hydrophobic substances for organic matter comprised in LF1 fraction. The weak association of hydrophobic compounds to the polar organic matter is responsible for their release to water during fractionation procedure (Krauss and Wilcke, 2002).

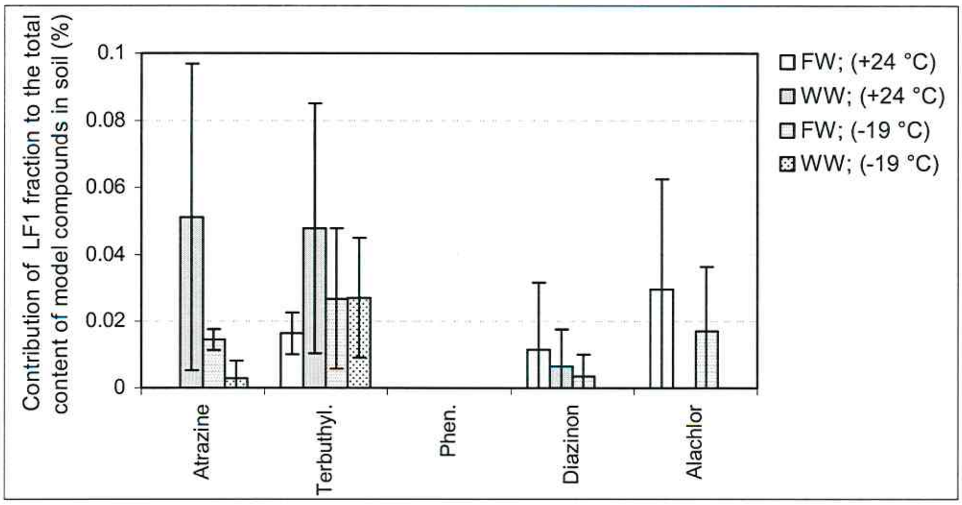


Figure 15. **Contribution of a specific light density fraction (LF1: < 1.8 g cm^{-3}) to the total content of model compounds (atrazine, terbuthyl. - terbuthylazine, alachlor, phen. - phenanthrene, diazinon) in soil; the fractions were isolated from soils of a citrus orchard at experimental site Bazra (Israel); FW - freshwater irrigated soil, WW - treated wastewater irrigated soil; soils after spiking with model compounds were 3 months aged at room temperature (+24 °C) and in refrigerator (-19 °C)**

4.3.4 The release of model compounds to the water during fractionation procedure

During isolation of clay fractions, the content of model compounds (for each model compound the initial amount was of 200 µg) released to the water directly after mixing with water and after each application of ultrasonic energy was once measured using SPME-GC-MS system.

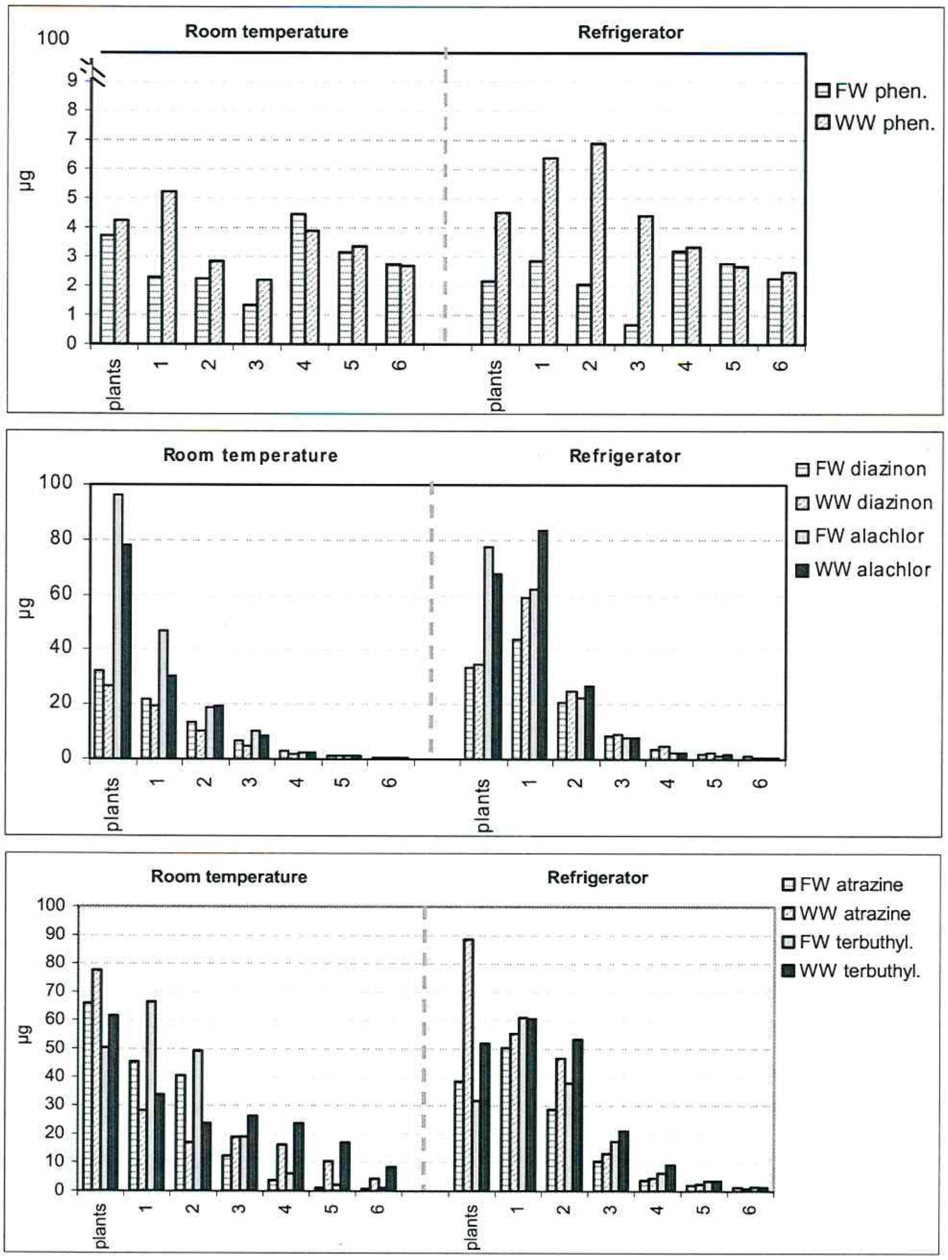


Figure 16. The release of model compounds (initial amount of each: 200 µg) from soils to the water phase, after flotation to separate plant residues (without ultrasonic energy treatment), and during fractionation procedure for isolating clay fractions (six ultrasonic treatments each 1 minute); soils were from a citrus orchard at experimental site Bazra; FW - freshwater irrigated soil, WW - treated wastewater irrigated soil; soils after spiking with model compounds were 3 months aged at room temperature (+24 °C) and in refrigerator (-19 °C); phen. - phehanthrene, terbuthyl. - terbuthylazine

The soils were treated 15 times with the ultrasonic energy of about 300 J mL^{-1}, each time for one minute. The water from each fractionation procedure was separated and the content of model compounds was determined.

The changes in content of model compounds in water samples stored from 7 to 15 treatments were negligible; therefore, these results were not presented. In figure 16, it can be demonstrated that each model compound had unique trend showing its release to the water during fractionation steps. The main differences were noticed for soils aged at the different temperatures. During fractionation, alachlor, diazinon, and atrazine were gradually released to the water from soils stored at the room temperature, whereas the trend for soils aged in refrigerator showed the breakthrough after 1-2 ultrasonic treatments. Excluding freshwater irrigated soil stored at the room temperature, the greatest proportion of terbuthylazine was indicated after first application of ultrasonic energy. Phenanthrene as the most hydrophobic compound presented irregular trend. The trends for freshwater and treated wastewater were not specific.

Although summary higher water solubility of model compounds in soils stored in refrigerator (excluding alachlor), the model compounds in these soils were slower released to the water during fractionation procedure. The increase in the temperature caused by ultrasonic treatment forced the release of model compounds to water. The solubility of model compounds at 25 °C reported in the literature increases in order: phenanthrene < terbuthylazine < atrazine < diazinon < alachlor (Table 3, Chapter 3.5). In this study the proportions of model compounds released during whole fractionation procedure increased as followed: phenanthrene < diazinon < atrazine = terbuthylazine < alachlor for soils stored at (+24°C), and phenanthrene < diazinon < alachlor < atrazine = terbuthylazine for soils stored at (-19°C) (Figure 17).

Atrazine and terbuthylazine having polar amine groups could form complexes with carboxyl group presence in LF1 fraction, and that can be the reason of their enhanced solubility. The diazinon and phenanthrene as the most hydrophobic substances were stronger associated to the soil, and despite application of ultrasonic energy, they remained in soil in quantities of 40-50% for diazinon and 50-70 % for phenanthrene.

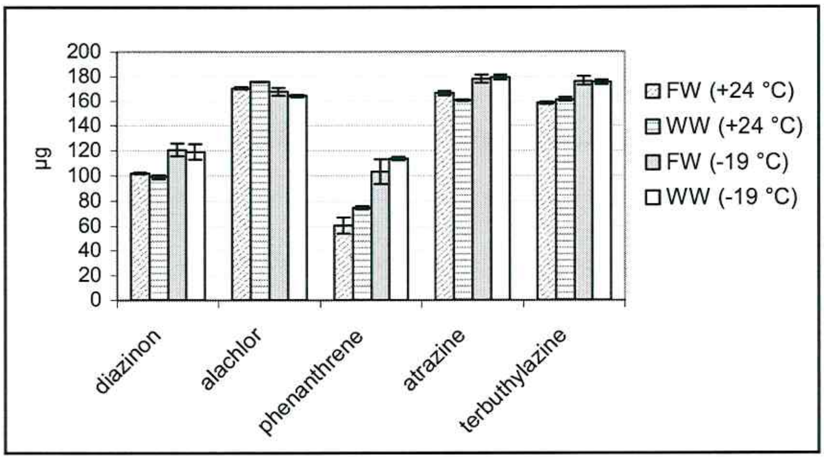


Figure 17. The total release of model compounds (initial amount 200 µg) to the water phase during fractionation procedure of soils of a citrus orchard at experimental site Bazra (Israel); FW - freshwater irrigated soil, WW - treated wastewater irrigated soil; soils after spiking with model compounds were 3 months aged at room temperature (+24 °C) and in refrigerator (-19 °C)

4.3.5 Conclusions

In chapter 4.2 the influence of wastewater irrigation and aging temperature of soil on distribution of atrazine, terbuthylazine, diazinon, alachlor, and phenanthrene among SOM fractions was tested. Aging of soil at low temperature resulted in limited diffusion processes and lower accumulation of hydrophobic substances in glassy SOM of clay fractions. Thus, higher proportions of hydrophobic substances in soils stored in refrigerator were accessible for extractants. The storage of the model compounds at room temperature enabled diffusion, mineralization, and formation of non-extractable residues of model compounds in soil.

The model compounds can be divided in three groups depending on which kind of bond with SOM was form: 1) phenanthrene and diazinon, 2) atrazine and terbuthylazine, 3) alachlor.

Atrazine, terbuthylazine and alachlor, which were in great amounts released to the water phase during fractionation steps, were weaker associated to SOM than phenanthrene and diazinon. Phenanthrene and diazinon as the most hydrophobic model compounds could be strongly bound to sorption sites through hydrophobic driving forces, or (π - π) interaction between the electron-donor ring and electron acceptor structural domains of SOM; therefore, they were poorly accessible for extractants.

The decomposition of organic matter in the SOM fractions which was deciding factor for adsorption of hydrophobic substances increased in order: LF1 < CF2 < CF1. The lower C/N ratio in fraction was indicated, the higher affinity for hydrophobic substances was detected. Phenanthrene and diazinon were not able to react with polar, carboxylic, and hydroxyl group representing a great proportion in low decomposable organic matter of LF1 fraction. Atrazine and terbuthylazine having polar amine groups could form complexes with carboxyl group presence in LF1 fraction, and that can be the reason of their enhanced solubility. Alachlor as a compound having the lowest hydrophobicity was found in great proportion in the fractionation water and its concentrations in SOM fractions were loaded with high error suggesting weakly and rather coincidental sorption on SOM fractions.

The wastewater irrigation did not significantly alter the sorption processes on SOM fractions.

4.4 Influence of irrigation water quality on sorption of model compounds on SOM fractions

In order to validate the results from distribution experiment, the batch sorption experiments with bulk soils, clay fractions, and bulk soil and clay minerals were conducted. The sorption isotherms were determined to understand the mechanisms ruling the sorption process of hydrophobic substances on soil and SOM fractions, and to reveal the changes in SOM caused by wastewater irrigation.

The sorption data collected from batch experiment were fitted to Freundlich, Langmuir and dual mode (DMM) models. Sorption pre-tests conducted with all model compounds on soils and clay fractions showed: 1) negligible sorption of atrazine, terbuthylazine and alachor, and 2) minor sorption of diazinon on soils and clay fractions. The low affinity of atrazine, terbuthylazine, and alachlor for SOM was already mentioned in the chapter describing distribution of model compounds in SOM fractions. Therefore, for batch experiments only phenanthrene which showed a strongest affinity for SOM was employed.

4.4.1 Development of a SPME-GC-MS method for sorption experiment

The SPME-GC-MS method was already used in the studies of sorption of hydrophobic substances on dissolved humic substances (HS), wastewater and DOM (Georgi and Kopinke, 2002; Ilani et al., 2003). It enabled direct determination of low concentrations of hydrophobic substances in dissolved organic matter solutions.

4.4.1.1 Optimization of solid-to-solution ratio

The solid samples were agitated for 6 hours in phenanthrene solution of 50 μg L^{-1} (Chapter 3.7.2).

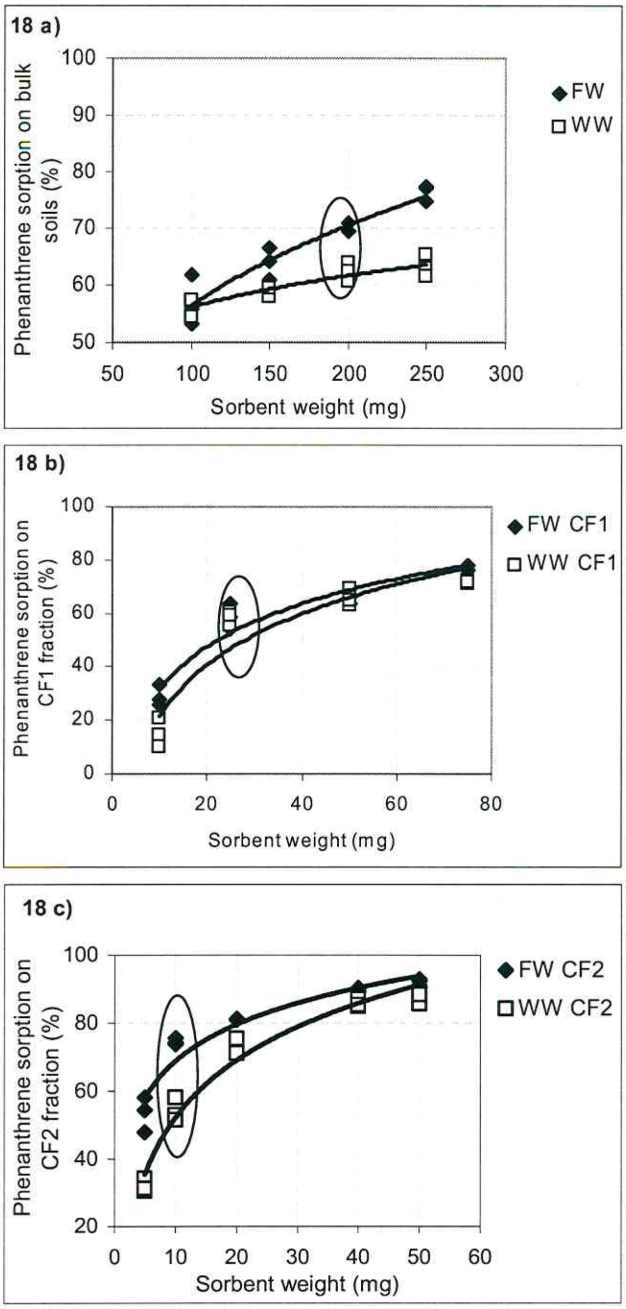


Figure 18. Optimization of solid-to-solution ratios for the determination of sorption isotherm of phenanthrene (initial concentration 50 µg L^{-1}); the sorbents used in experiment were: 18 a) bulk soils of a citrus orchard at experimental site Bazra (Israel) irrigated with freshwater (FW) and treated wastewater (WW); 18 b) CF1 fraction (< 1 µm), and 18 c) CF2 fraction (1-2 µm) isolated from these bulk soils

The tested solid/solution ratios were: 1:50; 1:30; 1:25; 1:20 (w:v) for bulk soils; 1:500; 1:200; 1:100, 1:66 (w : v) for CF1 fraction; and 1:1000; 1:500; 1:250; 1:125; 1:100 (w:v) for CF2 fraction (Appendix 6). Optimal solid/solution ratios: 1:25 (w:v) for bulk soils, 1:200 (w:v) for CF1 fraction, and 1:500 (w:v) for CF2 fraction, were settled for sorption between 60 % and 75 % of initial phenanthrene concentration (Figure 18a; 18 b; 18c). Although freshwater and treated wastewater irrigated soils had comparable contents of TOC (both about 0.7 %), the freshwater irrigated soil adsorbed larger amounts of phenanthrene than treated wastewater irrigated soil (Figure 18a; 18b; 18c). The high sorption to the CF2 fraction was caused by its carbon content, which was two times higher than that in CF1 fraction and eight times higher than that in bulk soils. The sorption increased nonlinear with an increasing solid/solution ratio. The nonlinear sorption can be attributed to the presence of DOM in the batch solution. After 6 h agitation of bulk soil samples in the 5 mL of 0.01 mol L^{-1} $CaCl_2$ solution, maximal 0.03 % of soil TOC was determined. Therefore, DOM could not cause the sorption nonlinearity. The clay fractions are known as a sink of stable organic matter strongly associated to mineral matrix; thus, the DOM content in experimental solutions was not detected (Gerzabek et al., 2001). The non-linear sorption can be caused by heterogeneous nature of SOM contained in soils and clay fractions. The affinity of phenanthrene for soil and clay matrices after destruction of organic matter using NaOCl (Chapter 3.3.2) was tested, and indicated as negligible due to hydratation of mineral surface. The sorption of phenanthrene was dependent only on composition and content of organic matter in sorbent material.

4.4.1.2 Optimisation of sorption time

In the literature, the equilibrium sorption time ranging from 24 h to 7 days for different sorption materials was reported (Zhou et al., 2004; Graber and Borisover, 2003).

Therefore, the phenanthrene uptake on soils and clay fractions after 6 h, 18 h, 48 h and 7 days of contact time was determined (Figure 19a; Figure 19b; Figure 19c.).

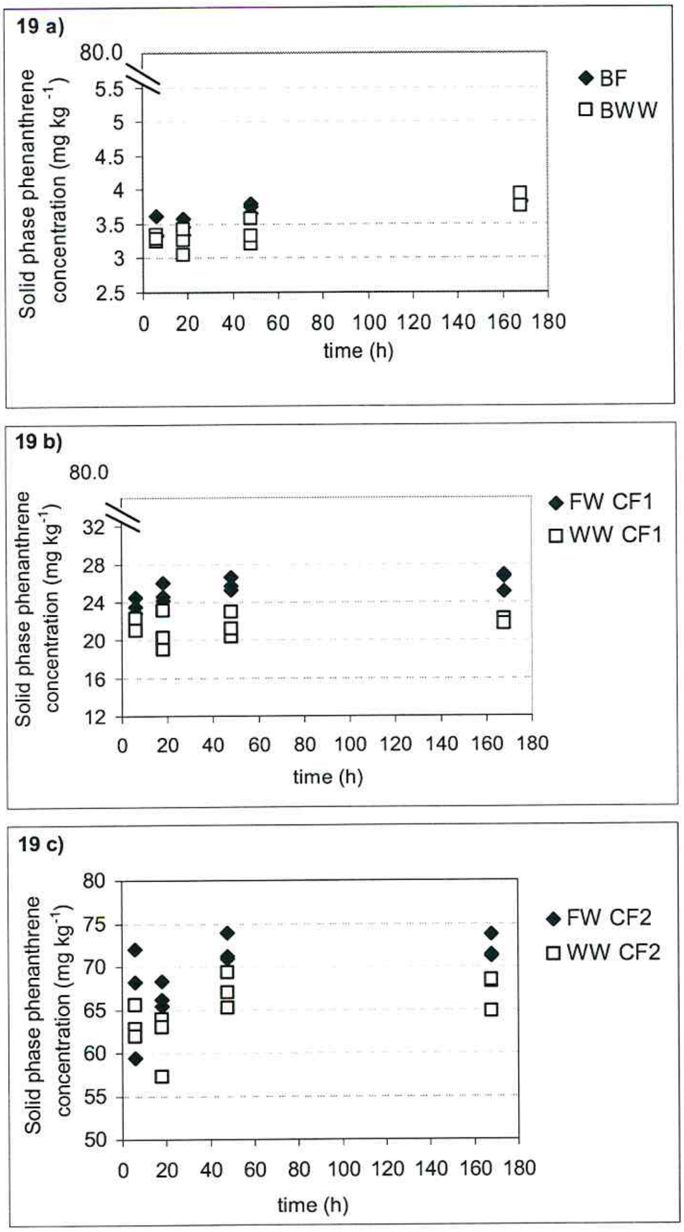


Figure 19. **The sorption of phenanthrene on different sorbents (calculated from phenanthrene uptake from solution; initial concentration was of 200 µg L^{-1}) presented as a function of sorption time; the sorbents used in experiment were: bulk soils of a citrus orchard at experimental site Bazra (Israel) irrigated with freshwater (FW) and treated wastewater (WW), CF1 fraction (< 1 µm), and CF2 fraction (1-2 µm) isolated from these bulk soils**

The initial phenanthrene concentration was 200 µg L^{-1}. The sorption experiments were conducted with: 200 mg of bulk soil, 25 mg of CF1 fraction, and 10 mg of CF2 fraction. There were no significant differences in phenanthrene uptake ($p < 0.05$) after 6, 18, 48 and 168 h of sorption time; therefore, the 6 h sorption time was chosen (Appendix 7). For the bulk soils and clay fractions the fast sorption process occurring within 6 h leaded to equilibrium.

4.4.2 Sorption isotherms and coefficients

4.4.2.1 Determination of model parameters

The equilibrium parameters resulting from fitting of Freundlich, Langmuir and DMM sorption isotherms to experimental data (Appendix 8) using OriginLab 7.5 software (OriginLab Corporation, Northampton, USA) are presented in Table 13, 14, 15, respectively (Equations 1, and 2; Chapter 2.4). They were obtained by weighted ($w = 1/q^2$) nonlinear regression (Lu and Pignatello, 2002; Zhao et al., 2001), where q (mg kg^{-1}) is the mass of adsorbed phenanthrene. The Marquardt-Levenberg and simplex algorithms were employed to find the best parameter, describing the data, for which the sum of the squares deviations of the theoretical curve from the experimental points for a range of independent variables is at its minimum (Zhao et al., 2001; Origin V75 User's Manual, OriginLab Corporation; Lebeouf and Weber, 2000). The chosen quantitative values used to describe how well the models fit the data, were mean weighted square error (MWSE), and adjusted R-square called also “goodness of fit” (r^2) (Xia and Ball, 1999).

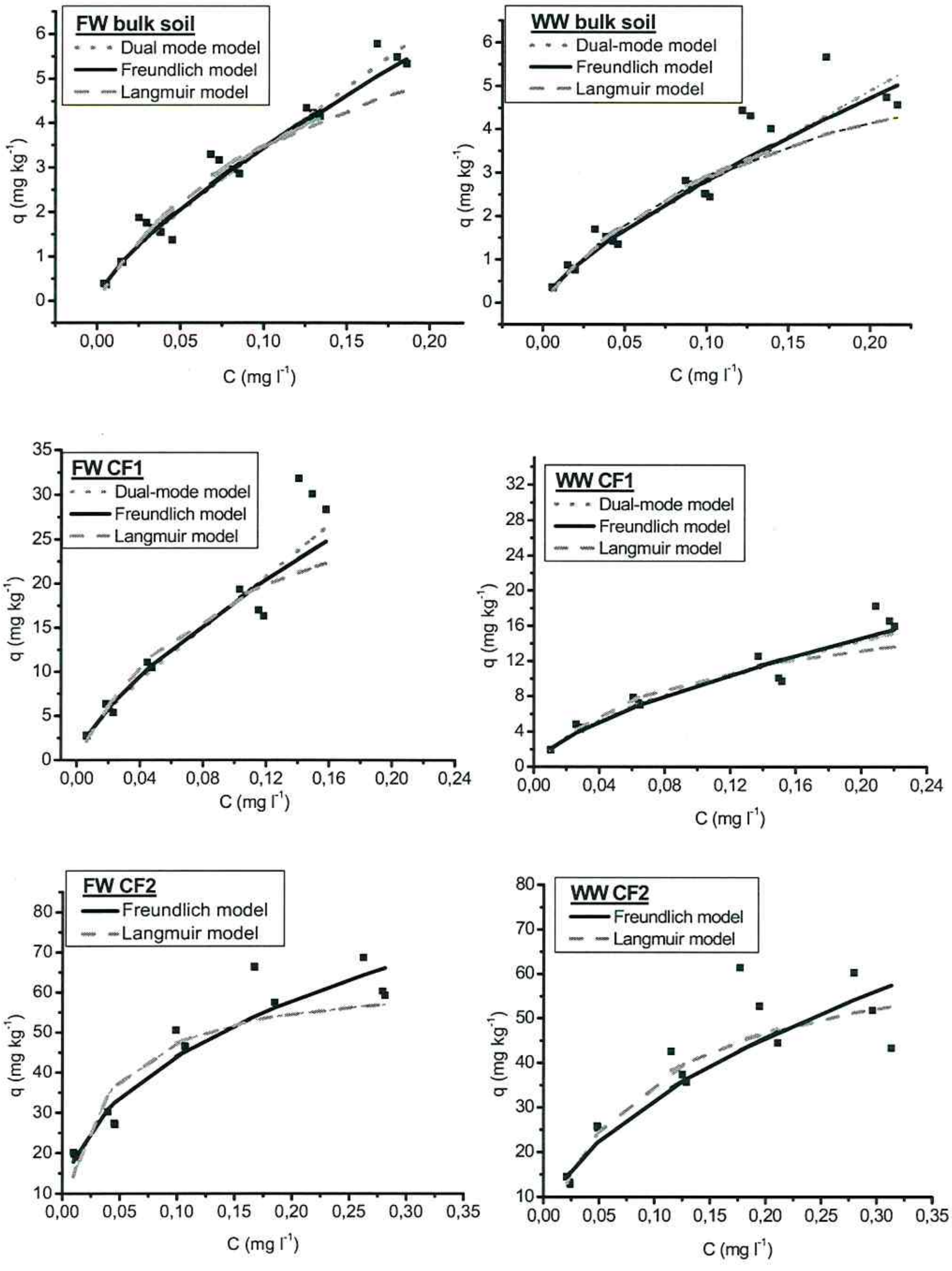


Figure 20. Langmuir, Freundlich and DMM isotherms fit to the experimental sorption data of phenanthrene on bulk soils of a citrus orchard at experimental site Bazra (Israel) irrigated with freshwater (FW) and treated wastewater (WW), and of clay fractions isolated from these bulk soils (CF1: < 1 μm; CF2: 1-2 μm); q - the amount of the solute sorbed per mass; C - an equilibrium solution concentration

The sorption isotherms of bulk soils and clay fractions were presented in Figure 20. The 24 experimental points for bulk soils irrigated with freshwater and treated wastewater and 15 experimental points for each CF1 and CF2 fractions were fitted to Freundlich, Langmuir and DMM sorption models (Figure 20). The paired sample T-test for dependent variables was applied to compare means of coefficients.

4.4.2.2 Fitting results for Freundlich isotherm

The Freundlich sorption model has been frequently used for the description of equilibrium and nonequilibrium sorption data from batch experiments and to predict degree of sorption nonlinearity (Lesan and Bhandari, 2003; Zhao et al., 2001). The sorption isotherms are presented in Figure 20. All parameters for Freundlich fit are summarised in Table 13.

Table 13. Freundlich isotherm coefficients for sorption of phenanthrene on bulk soils of a citrus orchard at experimental site Bazra (Israel) irrigated with freshwater (FW) and treated wastewater (WW) and on clay fractions isolated from these bulk soils (CF1: < 1 µm, CF2: 1-2 µm)

Freundlich model						
Samples	**K_F (1) (mg/kg) (mg/L)$^{-N}$**	**K_{OC} (2) (L kg^{-1})**	**logK_{OC}**	**N (3)**	**r^2 (4)**	**MWSE (5)**
FW bulk soil	18.50 ± 1.79	2513.0	3.400	0.727 ± 0.029	0.956	0.0239
WW bulk soil	15.61 ± 1.41	2364.7	3.374	0.740 ± 0.028	0.960	0.0218
FW CF1	89.44 ± 10.99	2702.1	3.432	0.696 ± 0.036	0.951	0.0234
WW CF1	41.89 ± 4.07	1878.3	3.274	0.653 ± 0.031	0.958	0.0188
FW CF2	108.33 ± 9.06	1787.7	3.252	0.390 ± 0.030	0.927	0.21
WW CF2	104.20 ± 12.14	2122.2	3.327	0.513 ± 0.046	0.895	0.39

[1] K_F (mg/kg) (mg/L)$^{-N}$ - the Freundlich equilibrium sorption coefficient

[2] K_{OC} = (100 %*K_F)/TOC - Freundlich equilibrium sorption coefficient normalised to carbon content in sorbent

[3] N - accounts for the degree of nonlinearity in the adsorption isotherm

[4] r^2 - R - square

[5] MWSE - mean weighted square error

Freundlich model gave satisfactory fit to bulk soils and CF1 fraction data; however, at higher solute concentrations, it overestimated q for CF2 fraction. It seems reasonable to notice that for CF2 fraction at the initial concentration above 200 mg L^{-1} the phenanthrene sorption presented plateau suggesting limited number of sorption sites assumed in Langmuir sorption model. Therefore, curves estimated for the sorption data of bulk soils and CF1 fraction differed from these of CF2 fraction.

Sorption coefficients (K_F) for phenanthrene ranged from 15.61 to 108.33 [(mg/kg) $(mg/L)^{-N}$] and were comparable to those presented by Zhou et al. (2004), and Lu and Pignatello (2002). The K_F increased in the order: **WW bulk soil < FW bulk soil < WW CF1 < FW CF1 < WW CF2 < FW CF2** (FW - freshwater irrigated soil; WW - treated wastewater irrigated soil). The K_F values were significantly higher for bulk soil irrigated with freshwater ($p < 0.01$) and its CF1 fraction ($p < 0.001$) in comparison to bulk soil irrigated with wastewater and its CF1 fraction. Moreover, the K_F values were strongly correlated with the TOC of soil/fractions ($r^2 = 0.927$; $p < 0.005$) (Figure 21). The normalization of K_F values to the carbon content in the sorbent generates K_{OC} coefficients and allows comparison of K_F values calculated from different sorption isotherms (Gunasekara and Xing, 2003). The normalization resulted in changes in the material's order: **FW CF2 < WW CF1 < WW CF2 < WW bulk soil < FW bulk soil < FW CF1**. The K_{OC} values displayed the differences between clay fractions of freshwater and treated wastewater irrigated soils and showed that adsorption on bulk soils was not significantly different when related to TOC of samples. Phenanthrene had significantly higher affinity (K_{OC}) ($p < 0.001$) for organic matter in CF1 fraction of freshwater irrigated soil than for the same fraction from treated wastewater irrigated soil (Table 13). The reverse relationship for the CF2 fraction was found. The results obtained for bulk soils suggested that the differences in sorption properties of clay fractions balanced each other. The sorption Freundlich parameter N varied between 0.390 and 0.740 and its values were inversely proportional to the TOC of sorbent samples ($r^2 = 0.871$; $p < 0.01$) (Figure 21). The higher was TOC of sorbent, the higher was the sorption nonlinearity due to the higher variety of sorption domains and sorption mechanisms. The lowest N values were calculated for the CF2 fraction containing organic matter of much more heterogeneous composition than that of CF1 fraction (Figure 10; Chapter 4.2.3). The composition of organic matter associated to 1-2 µm particles was closer to that of LF1 fraction having high content of polar groups, and low sorption capacity for hydrophobic substances (Figure 11, Chapter 4.2.4). The recently suggested reasons for sorption nonlinearity indicated as low N values were: 1) the hole filling mechanism caused by the presence of glassy SOM, 2) high aromaticity of organic matter, 3) aliphatic amorphous condensed conformation (Lu and Pignatello, 2002; Xing, 2001; Gunasekara and Xing, 2003; Salloum et al., 2002).

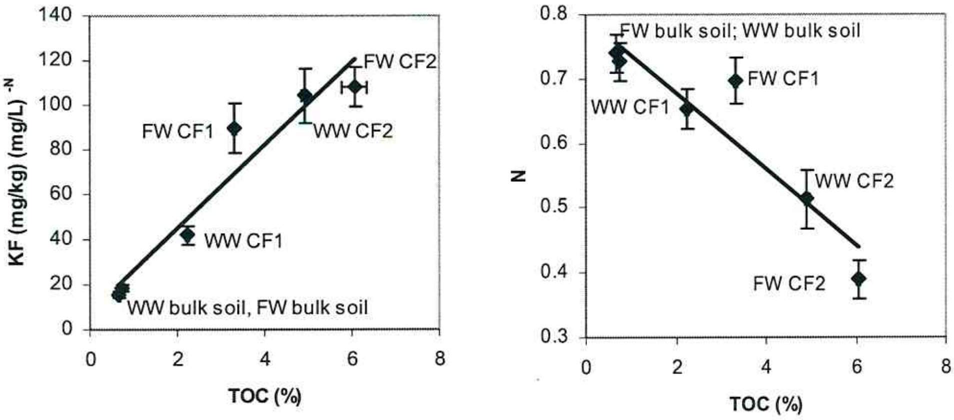


Figure 21. The correlation between KF, and N values and TOC in bulk soils of a citrus orchard at experimental site Bazra (Israel) irrigated with freshwater (FW) and treated wastewater (WW), and in clay fractions isolated from these bulk soils; FW - freshwater irrigated soil; WW - treated wastewater irrigated soil; CF1: < 1 µm; CF2: 1-2 µm; K_F - Freundlich equilibrium sorption coefficient (mg/kg) $(mg/L)^{-N}$; N - accounts for the degree of nonlinearity in the adsorption isotherm

Irrigation with treated wastewater increased the linearity of Freundlich sorption isotherms for CF2 fraction and did not influence the shape of sorption isotherms of bulk soils and CF1 fraction (Table 13).

The wastewater derived organic matter could influence the quality of organic matter in CF2 fraction by increasing of SOM proportion, which was not bound to the mineral matrix and therefore enriched dissolution domain of SOM having partitioning-like sorption mechanism.

4.4.2.3 Langmuir and DMM isotherms

All isotherms were characterized by high level of nonlinearity; therefore, the sorption data were also fitted to Langmuir sorption model as well as to DMM. Parameters are presented in Table 14 and 15.

Table 14. Langmuir isotherm coefficients for sorption of phenanthrene on bulk soils of a citrus orchard at experimental site Bazra (Israel) irrigated with freshwater (FW) and treated wastewater (WW) and of clay fractions (CF1: < 1 µm, CF2: 1-2 µm) isolated from these bulk soils

Langmuir model						
	Qmax (1) (mg kg^{-1})	**Q_{OC} (2) (mg kg^{-1})**	**Kd (3) (L mg^{-1})**	**Kd_{OC} (4) (L mg^{-1})**	**r^2 (5)**	**MWSE (6)**
FW bulk soil	7.87 ± 1.41	1069.3	8.15 ± 2.07	1107.3	0.926	0.0401
WW bulk soil	7.10 ± 6.49	1075.8	7.04 ± 9.06	1066.7	0.939	0.0333
FW CF1	36.02 ± 7.16	1088.2	10.28 ± 2.97	310.6	0.910	0.0424
WW CF1	19.49 ± 2.17	874.0	10.57 ± 1.85	474.0	0.953	0.0209
FW CF2	63.87 ± 6.72	1054.0	29.44 ± 7.86	485.8	0.811	0.0162
WW CF2	67.24 ± 5.72	1369.4	11.49 ± 1.87	234.0	0.938	0.0422

[1] Qmax - Langmuir capacity coefficient (mg kg^{-1})
[2] Q_{OC} = (100 %*Qmax)/TOC - Langmuir capacity coefficient (mg kg^{-1}) normalised to carbon content in sorbent material
[3] Kd - Langmuir affinity coefficient
[4] Kd_{OC} = (100 %*Kd)/TOC - Langmuir affinity coefficient normalised to carbon content in sorbent material
[5] r^2 - R-square
[6] MWSE - mean weighted square error

The equilibrium uptake at the high solute concentrations was underestimated by Longmuir isotherm; therefore, the coefficient r^2 values for Langmuir model are lower then that provided by dual mode and Freundlich models (Table 13, 14, 15 and Figure 20). These results were related to the conceptual limitation of Langmuir sorption model appearing when the adsorption capacity of tested materials exceeds the theoretically allowed monolayer coverage on SOM surface (Xia and Ball, 1999; Sander and Pignatello, 2005). The DMM at high solute concentrations assumes the plasticization of sorption sites and the liner sorption. This was observed for bulk soils and CF1 fraction but was missed in case of CF2 fraction (Figure 1; Lu and Pignatello, 2002). The higher solute concentration resulted in higher solute diffusion into sorption domains (Fick's low). It can be assumed that organic matter in CF2 fraction was dominated by glassy domains having hole-filling sorption mechanism. The presence of glassy SOM in this fraction was not accompanied by enhanced sorption predicted for such organic matter (Xia and Pignatello, 2001). Despite the greater amount of glassy SOM associated to CF2 fraction, its sorption capacity normalized to carbon content (Q_{OC}) was comparable to that presented for bulk soils and CF1 fraction. These results can be explained by size of phenanthrene molecules classified as large and excluded from adsorption on domains having microporous surface (Xia and Ball, 1999). Both K_F and Q_{max} parameters calculated using Freundlich and Langmuir models, respectively, displayed the same influence of wastewater organic matter on SOM in CF1 fraction because they are both proportional to the adsorbed mass of phenanthrene.

The DMM (Table 15) provided a good description of sorption of phenanthrene to CF1 fraction. However, this model did not fit to CF2 fraction sorption data. The ratios K_{DM} Q_{DM} b^{-1} calculated from the dual mode-model sorption parameters for dissolution (b) and glassy (K_{DM} and Q_{DM}) domains showed that the sorption to the organic matter in

CF1 fraction was based on hole filling mechanism (Langmuir model), whereas bulk soils showed greater dissolution domains sorption (partitioning model).

The CF1 fraction from treated wastewater irrigated soil presented slightly higher sorption on hole-filling domains.

Table 15. DMM isotherm coefficients for sorption of phenanthrene on bulk soils of a citrus orchard at experimental site Bazra (Israel) irrigated with freshwater (FW) and treated wastewater (WW) and of clay fractions (CF1: < 1 µm, CF2: 1-2 µm) isolated from these bulk soils

DMM						
	K_{DM} (1) ($L\ mg^{-1}$)	**Q_{DM} (2) ($mg\ kg^{-1}$)**	**b (3) ($mg\ L^{-1}$)**	**$K_{DM}\ Q_{DM}\ b^{-1}$ (4)**	**r^2 (5)**	**MWSE (6)**
FW bulk soil	98.7 ± 58.5	0.81 ± 0.31	27.0 ± 3.2	2.96	0.957	0.0246
WW bulk soil	61.8 ± 37.0	0.85 ± 0.37	20.6 ± 2.8	2.55	0.939	0.0333
FW CF1	151.5 ± 134.0	3.8 ± 1.7	143.8 ± 18.9	4.01	0.956	0.0226
WW CF1	28.5 ± 15.1	7.1 ± 3.2	41.0 ± 14.0	4.93	0.967	0.0167
FW CF2	NA(7)	NA	NA	NA	NA	NA
WW CF2	NA	NA	NA	NA	NA	NA

[1] K_{DM} - affinity coefficient of the hole-filling domain in DMM
[2] Q_{DM} - capacity coefficient of the hole-filling domain in DMM
[3] b - dissolution domain partitioning coefficient
[4] $K_{DM}\ Q_{DM}\ b^{-1}$ - DMM parameters ratio
[5] r^2 - R-square
[6] MWSE - mean weighted square error
[7] NA - model did not fit to CF2 fraction sorption data

4.4.3 Conclusions

The amount and properties of organic matter are perceived to be the major factors controlling sorption of phenanthrene on bulk soils and clay fractions. The batch sorption experiment evidenced and improved the assumptions from Chapter 4.3.

The Freundlich sorption coefficients of phenanthrene for soils irrigated with freshwater and treated wastewater were comparable due to composition of organic matter associated to clay fractions and not due to the similar carbon concentrations. The Freundlich parameters suggested that organic matter in CF1 fraction of freshwater irrigated soil had higher affinity for phenanthrene ($p < 0.001$) than that in CF1 fraction of treated wastewater irrigated soil. The sorption coefficients of CF2 fraction from freshwater and treated wastewater irrigated soils presented inverse relationship than these of CF1 fraction. The contrasted sorption properties of CF1 and CF2 fractions leaded to comparable sorption on freshwater and treated wastewater irrigated soils. The linearity of sorption isotherms (N parameter, Freundlich isotherm) was increased in the order: **FW CF2 < WW CF2 < WW CF1 < FW CF1< WW bulk soil < FW bulk soil**, and was inverse proportional to TOC of sorbent material. The quality of organic matter in clay fractions was of great importance in sorption processes on Bazra soils.

The DMM could satisfactory describe sorption of phenanthrene on organic matter of CF1 fraction and on bulk soils, whereas this model could not well fit the data points of CF2 fraction. Due to limited number of sorption sites in organic matter of CF2 fraction, its sorption data were better fitted to Langmuir than Freundlich or DMM sorption models. The ratios K_{DM} Q_{DM} b^{-1} showed that the sorption to the TOC of CF1 fraction was based on hole filling mechanism (Langmuir model), whereas bulk soils showed greater dissolution domains sorption (partitioning model). The sorption coefficients of CF1 fraction calculated from DMM enabled assumption that the irrigation with treated wastewater increased the amount of organic matter responsible for hole-filling sorption mechanism. This was related to the higher K_{DM} Q_{DM} b^{-1} ratio calculated for clay fractions of treated wastewater irrigated soil.

5 Summary

The deficit of water resources, growing population and the development of agriculture, and industry forced the usage of treated wastewater in the Middle East. The influence of sludge, wastewater and treated wastewater on soil physical and chemical properties was widely discussed. However, the number of studies describing the changes in SOM

fractions such as hot water extractable fraction, clay and density fractions is insufficient to asses the potential risk for mobilisation and immobilisation of organic pollutants in soil. There is a lack of information considering the stabilization of wastewater derived organic matter in soil. Furthermore, it is unknown how the alteration of SOM fractions influences the potential of organic matter for leaching of hydrophobic substances down in soil profile.

In this study soil samples from different soil layers (0-3, 3-30, 30-60, 60-90, and 90-120 cm) of a citrus orchard at Bazra site (costal plain of Israel) were tested. The samples from two different plots, long-term irrigated with freshwater and treated wastewater, were collected. Bulk soils as well as relevant SOM fractions were investigated for influence of wastewater irrigation on distribution and sorption of hydrophobic substances.

The SOM was fractionated into: a) decomposable pool represented by hot water extractable fraction (HWF); b) pool associated to clay fractions of soil (clay fractions: CF1: < 1μm and CF2: 1-2 μm); c) pool of specific light density fractions (LF1: < 1.8 g cm^{-3}, LF2: 1.8-2.0 g cm^{-3}) only loosely or not associated to soil minerals. The fractionation of SOM into the pools having different turn-over rates enables investigation of effect of wastewater originated organic matter on SOM system.

Both quality and quantity of SOM are essential for sorption of hydrophobic substances to soil and SOM fractions. Therefore, the interactions between five model compounds: atrazine, terbuthylazine, alachlor, diazinon, and pehanthrene and SOM fractions were studied in the experimental part of the work. The freshwater and treated wastewater irrigated soils were spiked with model compounds and aged at room temperature and in refrigerator. The experiment enables the investigation of 1) the distribution of hydrophobic substances among SOM fractions, 2) sorption capacities of clay and density fractions.

Basing on the results from distribution studies for a model hydrophobic compounds, phenanthrene was chosen for batch sorption experiment testing the sorption kinetic of hydrophobic substances on bulk soils and SOM fraction. The data sets from sorption experiment of bulk soils and clay fraction were fitted to Freundlich, Langmuir and dual-mode models to find the sorption mechanism appropriate for the sample's material, and to reveal the influence of wastewater irrigation on sorption coefficients.

The irrigation with wastewater caused an alteration of organic matter in bulk soil and SOM fractions. The wastewater derived organic carbon was predominantly contributed to TOC in the top soils indicated as significant increase in carbon concentrations in bulk soil, and all studied SOM fractions. The allocation of organic matter in soils irrigated with wastewater resulted in:

1) presence of the specific light density fraction at 60-90 and 90-120 cm soil depths,
2) significantly lower decomposition degree of organic matter comprised in CF1 fraction at 60-90 and 90-120 cm soil depths.

The wastewater derived polysaccharides and aromatic structures contributed to TOC of bulk soils and clay fractions in the top soil layer. In contrast, organic matter in LF1 was enriched in amides, carboxylic and hydroxyl groups. **The LF1 fraction might be a sink of wastewater organic matter** in soil due to an increase in polar group content in the upper soil layer, and their reduction in subsoil, where they can constitute the complexes with clay mineral surface.

The organic matter in hot water extractable fraction was altered during wastewater irrigation, which was indicated as significantly higher content of aliphatic domains and the low humification degree of organic matter in extracts obtained from 3-30 and 30-60 cm soil layers. It was the effect of competitive sorption of hydrophobic organic matter on soil minerals and elution of hydrophilic organic matter down in soil horizon.

The distribution of model compounds in SOM fractions enables their classification in three groups:

1) penanthrene and diazinon, strongly hydrophobic compounds constituting the hydrophobic driving forces or (π - π) bonds between the electron-donor ring and electron acceptor structural domains of organic matter in clay fraction,
2) atrazine and terbuthylazine forming donor-acceptor transfer of (π - π) electrons as well as ionic bonds with carboxylate groups of SOM,
3) alachlor having higher affinity for polar water molecules than for SOM.

In Bazra soils phenanthrene and diazinon were able to form bound residues decreasing their availability for extractants; however, these hydrophobic compounds were not able to constituent bonds with polar groups contained in organic matter of LF1 fraction. Atrazine and terbuthylazine had higher affinity for polar groups of "fresh organic matter" and were easily leached to the water during fractionation steps.

Generally, organic matter contained in CF1 fraction had higher affinity for hydrophobic substances than that contained in CF2 fraction due to:

1) the higher decomposition degree,
2) high proportions of aliphatic groups,
3) low proportions of N containing domains.

Soil freezing resulted in limited diffusion process of hydrophobic substances enhancing their extractability, and suppressing formation of bound residues.

Batch sorption experiments evidenced the assumption made for the distribution of hydrophobic substances in SOM fractions. The sorption of phenanthrene on soils and clay fractions was controlled by carbon content due to the hydratation of mineral surface. The Freundlich isotherm was well fitted to sorption data for bulk soils and clay fractions indicating:

1) higher affinity of CF1 fraction isolated from freshwater irrigated soil for phenanthrene in comparison with CF1 fraction from treated wastewater irrigated soil,
2) inverse relationship for CF2 fraction isolated from the same soil.

In bulk soils irrigated with freshwater and treated wastewater the contrasting sorption properties of clay fractions gave in effect comparable sorption coefficients. The sorption linearity characterized by N parameter calculated from Freundlich isotherm was increased in order: **FW CF2 < WW CF2 < WW CF1 < FW CF1 <WW bulk soil < FW bulk soil** and was inverse proportional to TOC in sorbent material.

The phenanthrene sorption data for CF2 fraction were better fitted to the Langmuir isotherm although a slight underestimation. Thus, the organic matter in this fraction was assumed to have limited number of sorption sites. Although higher sorption capacity, the CF2 fraction sorbed less phenanthrene than CF1 fraction due to:

1) lower number of sorption sites,
2) higher polarity of organic matter related to the high content of amid groups indicated in DRIFT spectra,
3) the exclusion of large phenanthrene molecules from adsorption on domains having microporous surface.

The wastewater irrigation increased sorption linearity suggesting contribution of partitioning-like domains to organic matter of CF2 fraction.

The dual mode model could well describe sorption points of phenanthrene for bulk soils and CF1 fraction, but failed in fitting of CF2 fraction sorption data. Due to the similar sorption trend of bulk soil and CF1, it can be reasonable to assume that CF1 fraction ruled the sorption processes at Bazra soil. This fraction comprises glassy organic matter which sorption holes could be swelled at the high solute concentrations forcing to sorption linearity.

6 Evaluation of the results and a need for future research

The results presented a substantial expend of the knowledge of the influence of irrigation water quality on SOM fractions, and how the alteration of organic matter influences the interaction between SOM fraction and hydrophobic compounds.

For the first time influence of treated wastewater on hot water extractable, clay and density fractions was studied using spectroscopic methods. The wastewater irrigation caused the alteration of hot water extractable, clay and density fractions leading to allocation of organic matter down in soil profile. Wastewater originated organic matter eluted hydrophilic organic matter from the mineral surface down in soil profile. The characterization of specific light density fraction LF1 evidenced its essential role in mobilisation, and immobilisation of pollutants in soil.

The irrigation with wastewater constitutes a risk for environment due to decrease in sorption affinity of hydrophobic substances for clay fractions. This could be a reason of presence of hydrophobic substances deep in soil profile.

In order to validate the results, investigation with soils having different texture and elemental composition could further extend the knowledge about the changes in SOM fractions caused by wastewater irrigation. The usage of ^{15}N and ^{13}C as tracer in hydrophobic substances and the evaluation of sorption experiments in column system could provide the further information about the biochemical processes and metabolism of hydrophobic substances in soil.

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10 Appendix

Appendix 1. Mass balances of particle size (CF1: < 1 µm, CF2: 1-2 µm), density fractions (LF1: < 1.8 g cm^{-3}, LF2: 1.8-2.0 g cm^{-3}) and fractionation rest (FR) of soils from the different soil layers of a citrus orchard at experimental site Bazra (Israel) long-term irrigated with freshwater and treated wastewater

Soil depth	Fraction mass (g)						
	Plants	CF1	CF2	LF1	LF2	FR	Sum Total
	Freshwater irrigated soil						
0-3	0.299 (0.118)	2.916 (0.079)	1.673 (0.049)	0.778 (0.027)	0.550 (0.132)	13.31 (0.19)	19.53
3-30	0.060 (0.013)	1.996 (0.048)	0.747 (0.043)	0.064 (0.012)	0.115 (0.021)	16.84 (0.09)	19.82
30-60	0.029 (0.05)	2.140 (0.082)	0.624 (0.059)	0.017 (0.001)	0.022 (0.003)	16.99 (0.04)	19.82
60-90	0.018 (0.002)	2.746 (0.029)	0.593 (0.032)	NA	0.026 (0.013)	16.39 (0.05)	19.78
90-120	0.018 (0.002)	3.098 (0.039)	0.628 (0.082)	NA	0.017 (0.005)	16.09 (0.04)	19.86
	Treated wastewater irrigated soil						
0-3	0.510 (0.206)	2.277 (0.107)	1.452 (0.052)	1.056 (0.095)	0.685 (0.431)	13.58 (0.586)	19.56
3-30	0.032 (0.006)	2.610 (0.035)	0.837 (0.054)	0.048 (0.005)	0.206 (0.171)	16.06 (0.17)	19.79
30-60	0.019 (0.003)	3.336 (0.018)	1.062 (0.109)	0.038 (0.005)	0.213 (0.200)	15.17 (0.21)	19.84
60-90	0.042 (0.042)	3.727 (0.093)	1.088 (0.066)	0.027 (0.001)	0.078 (0.067)	14.79 (0.09)	19.75
90-120	0.015 (0.003)	3.902 (0.038)	0.948 (0.024)	0.017 (0.006)	0.041 (0.017)	14.86 (0.05)	19.78

NA - not available, no LF1 fraction was isolated

Appendix 2. Spectroscopic characterization of hot water extractable fraction (HWF) isolated from soil samples of a citrus orchard at experimental site Bazra (Israel) long-term irrigated with freshwater and treated wastewater

		UV-VIS Absorbances			Areas calculated from emission fluorescence spectra	
Soil depth (cm)	Carbon concentration in HWF (mg L^{-1})	Absorbance at 280 nm	Absorbance at 465 nm	Absorbance at 665 nm	Area between 300-345 nm	Area between 435-480 nm
	Freshwater irrigated soil					
0-3	478 (11)	3.51 (0.19)	0.436 (0.013)	0.035 (0.001)	226 (9)	1257 (45)
3-30	72.0 (5.7)	1.76 (0.10)	0.107 (0.001)	0.007 (0.000)	170 (52)	2751 (1002)
30-60	30.4 (4.0)	0.61 (0.09)	0.027 (0.002)	0.003 (0.000)	255 (21)	4534 (472)
60-90	19.0 (0.4)	0.36 (0.01)	0.019 (0.000)	0.003 (0.000)	204 (16)	3787 (401)
90-120	17.1 (0.5)	0.31 (0.01)	0.015 (0.000)	0.002 (0.001)	219 (24)	3792 (502)
	Treated wastewater irrigated soil					
0-3	439 (20)	3.20 (0.09)	0.463 (0.007)	0.034 (0.002)	237 (5)	1244 (13)
3-30	58.3 (0.7)	1.33 (0.04)	0.072 (0.001)	0.002 (0.000)	194 (20)	2819 (290)
30-60	31.2 (1.8)	0.59 (0.02)	0.027 (0.001)	0.001 (0.000)	226 (27)	3474 (355)
60-90	24.9 (0.6)	0.44 (0.00)	0.026 (0.001)	0.005 (0.001)	260 (7)	3636 (507)
90-120	21.8 (0.7)	0.35 (0.01)	0.018 (0.003)	0.002 (0.001)	253 (7)	2936 (147)

Appendix 3. **The average recoveries (n = 3) of model compounds (atrazine, terbuthylazine, alachlor, diazinon, phenanthrene) in soils of a citrus orchard at experimental site Bazra (Israel); FW - freshwater irrigated soil, WW - treated wastewater irrigated soil; soils after spiking with model compounds were 3 months aged at room temperature (+24 °C) and in refrigerator (-19 °C)**

	Recovery µg g^{-1}				
	Atrazine	Terbuthylazine	Alachlor	Diazinon	Phenanthrene
FW (+24 °C)	9.076 (0.523)	9.001 (0.238)	9.286 (0.660)	5.998 (0.193)	7.069 (0.250)
WW (+24 °C)	8.201 (0.939)	8.958 (0.912)	9.711 (0.232)	5.786 (0.116)	7.401 (0.535)
FW (-19 °C)	9.649 (0.928)	9.763 (0.191)	9.072 (0.283)	7.378 (0.393)	9.092 (0.373)
WW (-19 °C)	9.704 (0.604)	9.699 (0.581)	9.013 (0.221)	7.537 (0.171)	8.948 (0.284)

Dry matter of FW soil was 98 % and that of WW soil was 97.5 %

Appendix 4. **The average concentrations (n=3) of diazinon, alachlor and phenanthrene, in plant residues, clay fractions (CF1: < 1 µm, CF2: 1-2 µm), density fraction (LF1: < 1.8 g cm^{-3}) from soils irrigated with freshwater (FW) and treated wastewater (WW) of a citrus orchard at experimental site Bazra (Israel); soils after spiking with model compounds were 3 months aged at room temperature (+24 °C) and in refrigerator (-19 °C), respectively**

Fraction	**Dry matter**		**Concentration of model compound measured in SOM fraction µg g^{-1}**					
			Diazinon		Alachlor		Phenanthrene	
	Room temperature							
	FW	**WW**	**FW**	**WW**	**FW**	**WW**	**FW**	**WW**
Plants	91.5	90.2	64.3 (6.3)	49.2 (0.9)	124 (10)	108 (29)	154 (18)	177 (3)
CF1	89.0	90.0	5.26 (0.70)	3.65 (1.20)	2.9 (0.2)	2.2 (1.3)	26.8 (2.5)	18.0 (0.9)
CF2	89.0	90.7	4.20 (0.42)	2.79 (1.08)	2.4 (0.0)	1.42 (1.50)	27.9 (1.9)	19.8 (1.8)
LF1	86.6	86.6	0.236 (0.410)	0.150 (0.259)	0.945 (1.050)	NA	NA	NA
	Refrigerator							
	FW	**WW**	**FW**	**WW**	**FW**	**WW**	**FW**	**WW**
Plants	91.5	91.5	57.7 (17.6)	90.6 (9.2)	73.6 (9.5)	84.0 (13.0)	89.7 (15.6)	173.7 (50.7)
CF1	90.6	88.8	8.8 (2.2)	8.7 (2.3)	2.70 (0.68)	3.21 (0.68)	26.4 (3.6)	16.3 (1.0)
CF2	93.6	91.4	6.0 (1.4)	5.26 (0.92)	2.16 (0.47)	2.38 (0.13)	27.4 (2.5)	18.7 (2.2)
LF1	86.6	86.6	0.091 (0.158)	NA	0.521 (0.584)	NA	NA	NA

NA - Not available (signal was not detectable)

Appendix 5. **The average concentrations (n=3) of atrazine and tarbuthylazine in plant residues, clay fractions (CF1: < 1 µm, CF2: 1-2 µm), density fraction (LF1: < 1.8 g cm^{-3}) isolated from soils irrigated with freshwater (FW) and treated wastewater (WW) of a citrus orchard at experimental site Bazra (Israel); soils after spiking with model compounds were 3 months aged at room temperature (+24 °C) and in refrigerator (-19 °C), respectively**

Fraction	**Dry matter**		**Concentration of model compound measured in SOM fraction** µg g^{-1}			
			Atrazine		Terbuthylazine	
	Room temperature					
	FW	**WW**	**FW**	**WW**	**FW**	**WW**
Plants	91.5	87.8	199 (26)	335 (12)	138 (23)	272 (6)
CF1	92.0	87.8	1.73 (0.11)	0.874 (0.265)	4.15 (0.45)	2.05 (0.51)
CF2	91.2	91.3	1.60 (0.74)	1.65 (0.39)	4.19 (2.43)	4.14 (0.81)
LF1	89.1	86.6	NA	2.45 (2.14)	0.708 (0.208)	2.69 (2.03)
	Refrigerator					
	FW	**WW**	**FW**	**WW**	**FW**	**WW**
Plants	91.5	87.8	247 (64)	284 (43)	204 (32)	257 (16)
CF1	86.8	88.1	1.40 (0.21)	1.04 (1.25)	3.89 (0.58)	2.53 (0.30)
CF2	87.4	90.5	1.20 0.61)	1.00 (0.07)	2.98 (1.20)	2.95 (0.19)
LF1	86.6	86.6	0.580 (0.161)	0.1309 (0.227)	1.16 (0.97)	1.35 (0.96)

NA - Not available (no signal in extracts was detected)

Appendix 6. **The concentrations of phananthrene in $CaCl_2$ solution after 6 h agitation at the different sorbent-to-solution ratios; the tested sorbents were: bulk soils of a citrus orchard at experimental site Bazra (Israel) irrigated with freshwater (FW) and treated wastewater (WW); CF1 fraction (< 1 µm), and CF2 fraction (1-2 µm) isolated from these bulk soils; initial concentration was of 50 µg L^{-1}**

	The concentration of phenanthrene in $CaCl_2$ solution (µg L^{-1})							
sorbent weight (mg)	bulk soil		sorbent weight (mg)	CF1 fraction		sorbent weight (mg)	CF2 fraction	
	FW	WW		FW	WW		FW	WW
100	21.5 (2.2)	21.9 (0.8)	10	35.6 (1.9)	42.4 (2.7)	5	23.3 (2.6)	34.0 (1.0)
150	18.1 (1.3)	20.5 (0.4)	25	20.8 (2.3)	20.8 (1.5)	10	12.7 (0.4)	22.9 (1.7)
200	15.0 (0.5)	18.9 (1.2)	50	16.8 (1.8)	17.1 (1.6)	20	9.5 (0.1)	13.0 (1.2)
250	11.8 (0.7)	18.4 (1.0)	75	11.4 (0.6)	14.1 (0.3)	40	5.2 (0.3)	7.1 (0.5)
						50	4.0 (0.6)	6.1 (0.9)

Appendix 7. **The concentrations of phenanthrene (µg L^{-1}) in $CaCl_2$ solution after 6, 18, 48, 168 h of agitation with different sorbates: 200 mg bulk soils of a citrus orchard at experimental site Bazra (Israel) irrigated with freshwater (FW) and treated wastewater (WW), 25 mg of CF1 fraction (< 1 µm) and 10 mg of CF2 fraction (1-2 µm) isolated from these bulk soils; initial solute concentration was of 200 µg L^{-1}**

Sorbent	**Sorption time (min)**			
	6	**18**	**48**	**168**
FW bulk	63.1 (6.3)	61.5 (4.7)	50.7 (2.9)	47.1 (0.4)
WW bulk	68.5 (1.)	70.3(7.7)	65.2 (7.7)	47.3 (3.6)
FW CF1	82.0 (4.2)	75.3 (4.8)	70.8 (3.4)	68.9 (5.2)
WW CF1	90.9 (3.3)	96.0 (10.6)	92.3 (6.6)	89.9 (1.4)
FW CF2	66.8 (12.9)	66.8 (3.1)	56.0 (3.3)	55.7 (2.9)
WW CF2	73.1 (3.9)	77.2 (7.1)	65.6 (4.1)	65.7 (4.2)

Appendix 8. The concentrations of phenanthrene (µg L^{-1}) in $CaCl_2$ solution after 6 h of agitation with different sorbates: 200 mg bulk soils of a citrus orchard at experimental site Bazra (Israel) irrigated with freshwater (FW) and treated wastewater (WW), 25 mg of CF1 fraction (< 1 µm) and 10 mg of CF2 fraction (1-2 µm) isolated from these bulk soils; initial concentrations were: from 20 to 400 mg L^{-1} for bulk soils, from 20 to 300 mg L^{-1} for CF1 fraction (< 1µm), from 50 to 400 mg L^{-1} for CF2 fraction (1-2 µm)

Sorbent	**Initial concentrations of phenanthrene (µg l^{-1})**					
	20	50	100	200	300	400
FW bulk	4.6 (0.7)	15.0 (0.5)	35.0 (7.0)	77.0 (8.2)	129.9 (4.1)	178.2 (9.0)
WW bulk	6.0 (0.4)	17.6 (2.3)	40.2 (4.9)	96.2 (5.9)	129.4 (8.9)	199.9 (23.5)
	20	50	100	200	300	
FW CF1	6.3 (0.3)	20.8 (2.5)	46.5 (1.5)	112.3 (8.0)	153.7 (6.1)	
WW CF1	10.2 (0.2)	27.3 (1.4)	63.1 (2.2)	146.0 (7.7)	215.3 (5.6)	
	50	100	200	300	400	
FW CF2	10.7 (1.1)	43.6 (3.4)	104.2 (4.6)	172.8 (10.3)	282.6 (10.6)	
WW CF2	22.9 (1.7)	48.7 (0.4)	119.7 (8.2)	169.1 (17.0)	299.1 (13.0)	

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