The Study of Fate and Mobility of Oxytetracycline and Doxycycline in Soil Column Matrices

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Abstract

Pharmaceutical pollution is one of the most serious types of environmental pollution attracting increasing attention and leading research studies in recent years. Because of their great impact on aquatic life, soil and underground water as emerging aquatic micro pollutants, it's possible that they have been affecting the ecological system. In this study, two antibacterials, oxytetracycline and doxycycline were selected as examples of pharmaceuticals that are released into the environment and have major health impacts on our life, such as allergic reactions in the body, hives; difficulty in breathing. Both are marketed in Palestine either for the human pharmaceutical industry or the veterinary one. In this research, the adsorption behavior of both pharmaceuticals on soil, the effect of organic matter, the effect of magnesium chloride hepta hydrate addition on polluted soil, and their effect on characteristics of underground water, were all studied using the UV-Vis spectrophotometer. The results showed that increasing organic matter increases the adsorption of oxytetracycline more than doxycycline, and that the composition of oxytetracycline complex with magnesium ion was more stable than that of doxycycline complex with magnesium. The study also revealed a higher concentration of doxycycline in leachate water from the soil than that of oxytetracycline, because doxycycline has higher solubility in water. It also showed a decrease in the concentrations for both substances over time in leachate water due to degradation. The degradation of both pharmaceuticals in soil and water would be produced by other substances which may be harmful, as the threat of their presence in the soil and groundwater would increase the resistance of bacteria in the soil. In other words, that would affect the natural properties of soil and groundwater, as well.

Keywords: Doxycycline; Leachate; Column; Isotherm; Oxytetracycline; Wastewater.

Introduction

Pharmaceuticals are becoming an emerging environmental issue that has attracted increasing attention in recent years, as emerging aquatic micro pollutants have possibly been affecting the ecological system. These compounds used by humans and livestock are mainly excreted through urine in an unaltered or altered form. Prescription drugs such as hormones, corticosteroids and antibiotics are showing up in our ground water, soil, waterways and even in our drinking water, with or without metabolism; they are later released into the aquatic system [1]. This so-called "pharmaceutical pollution", could have major implications on wildlife, agriculture,

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humans and yet is only beginning to be studied. That's because our conventional sewage treatments may not be looking for drugs, and certainly don't always remove them [2, 3].

The presence of pharmaceutical compounds in treated wastewater and in surface waters is a growing environmental concern. Treated wastewater is the primary mechanism by which pharmaceuticals are introduced to the environment[3]. Only a fraction of medication is completely absorbed by the body, and the excess is excreted as unchanged compounds or processed metabolites. With septic systems, pharmaceutical compounds leach directly into ground water[1]. With municipal sewage, the compounds make their way to sewage treatment facilities that are not equipped to degrade medicinal substances. The result is wastewater effluent that contains various degrees of pharmaceutical waste, much of which goes undetected because water districts and sewage treatment facilities are not required to be tested for pharmaceuticals[3].

Among various kinds of pharmaceuticals, antibiotics were more frequently detected than others. They are difficult to remove through common biological treatment methods.

According to recent research, a variety of antibiotics were detected in various water samples including hospital wastewater, municipal wastewater, effluent of wastewater treatment plants, antibiotics industry wastewater, livestock farm mud and wastewater, surface water, underground water and drinking water[2].

This study chooses Doxycycline (DOX) and Oxytetracycline (OTC) as an example of tetracyclines that are released into the environment. The research investigated their adsorption onto soil, the effect of organic matter on their adsorption onto soil. The effect of magnesium chloride hepta hydrate addition on polluted soil was also studied. In addition the effect of OTC and DOX on pH of leachate water before and after pollution and their concentrations in leachate water were measured versus time. Oxytetracycline and Doxycycline were chosen because of their wide application here in Palestine, high-solubility in water and high residual toxicity.

Locally, both OTC and DOX are used in pharmaceutical manufacturing products, especially in the local veterinary sector; since they are manufactured under many local trade names, such as Oxin 50%, Doxinal 10%. OTC and DOX belong to tetracycline antibiotics that are indicated to treat infections caused by gram positive and gram negative bacteria. According to the Palestinian Ministry of Health tons of antibiotics are consumed every year. The average consumption in both human and veterinary sectors reached 2.6 tons of doxycycline HCl and 4.5 tons of oxytetracycline HCl in the last two years.

Materials and Methods

Experimental Work

The experimental work in this research depended basically on determining the concentration of residues of oxytetracycline HCl and doxycycline HCl versus time in soil and leachate water (in which it was considered here as the underground water) after 24 hours of adsorption. Samples of soil and leachate water were analyzed by UV-Vis spectrophotometer at different periods of time at constant temperature. In addition, the effect of $MgCl_2$ addition on soil was studied as well. The room temperature recorded ranged between 18°C - 22°C. Each measurement in this study was the average of three readings to ensure that consistent values were obtained. All the glassware used was cleaned and dried before each measurement. Standard readings were obtained for oxytetracycline HCl and doxycycline HCl and plotted against absorbance readings, in order to calculate the concentrations of both substances in soil and leachate water.

Soil Column Preparation

In this study seven soil columns were prepared from PVC plastic, the dimensions were 1.0 m long and 15.2 cm in diameter, and the soil was gathered from 600 m^2 area located on the top of Mount Gerizim in Nablus city. The location was far away from any expected source of contamination with any type of pharmaceuticals. Randomly, and from different sites, the soil was collected, mixed and filled inside the columns; two kilograms were taken and sieved for the soil analysis before any treatment. The soil columns were then washed with distilled water to ensure that the pH of outgoing water from each column was neutral.

Soil Analysis

The soil used for chemical analysis was sieved in a 2.0 mm sieve, and dried at 105°C. Several tests were conducted on the soil before any treatment with pharmaceuticals such as soil texture, pH, total nitrogen and organic matter. All results are shown in table 1 and the tests were conducted three times for each sample.

Table 1: Soil texture, moisture content, moisture correction factor, pH, organic carbon, organic matter and nitrogen present for soil before pollution

Test	Result
Soil Texture	71.6% clay, 6.16% silt, 22.24% sand
Moisture	2.6%
Moisture correction factor (mcf)	1.026 ± 0.05
pH	7.13 ± 0.3
Organic Carbon %	2.45% ±0.1
Organic Matter %	4.21% ±0.3
Nitrogen Content	0.155%

Calibration Curves

Standard calibration curves for both oxytetracycline and doxycycline were performed by preparing diluted solutions of oxytetracycline HCl and doxycycline HCl standards. Both were purchased from KEMPEX, Holland.

A 100.0 mg of oxytetracycline HCl reference standard and 100.0 mg of doxycycline HCl reference standard were accurately weighed (each of which alone), then transferred into 100 ml volumetric flasks. Distilled water was added to the volume and stirred until completely dissolved. Several dilutions were made from the solution by taking 1.0, 2.0, 3.0, 4.0 and 5.0 mL samples from stock solution and transferring them into 50 mL volumetric flasks. Distilled water was added to volume. Absorbance readings were recorded at 353 nm for oxytetracycline HCl and at 270 nm for doxycycline HCl.

Optimum Time for Oxytetracycline and Doxycycline Adsorption onto Soil

The purpose of this task was to determine the optimum time for the process of adsorption of both oxytetracycline HCL and doxycycline HCl onto soil to reach equilibrium. Two samples of oxytetracycline HCl solution and another two samples of doxycycline HCl were prepared in 125 ml Erlenmeyer flask containing 5 grams of oven dried sieved soil, and 50 ml of 0.005% (w/v) of each tetracycline solution. All samples were covered with Teflon screw caps and mounted on Comfort Hetro Master Shaker at room temperature. All samples were kept for 1, 2, 4, 6, 12, 24 and 36 hr intervals. Soil particles were allowed to settle then be centrifuged using Hermel Z200A Centrifuge set at 3000 rpm for 10 mins. After centrifuging, absorbance readings were recorded at 353 nm for oxytetracycline HCl and at 270 nm for doxycycline HCl using UV-1601 PC, SHIMADZU spectrophotometer. It should be mentioned that two samples were prepared for each pharmaceutical in order to confirm the results.

Isotherms

The most widely used equation to fit empirical data from solute – solvent adsorbent system is the Freundlich equation. Due to its simplicity and versatility in fitting data from systems, Freundlich's relationship will be used in this study to describe the quantitative adsorption of tetracyclines onto soil.

Six different concentrations 0.003%, 0.004%, 0.006%, 0.008%, 0.01% and 0.012% (w/v) each of oxytetracycline HCl and doxycycline HCl solutions were prepared, each in 125 ml Erlenmeyer flask; 5.0 grams of oven-dried sieved sample were added to each flask, and 50 ml of each concentration for each substance were added to each flask. All samples were covered with Teflon screw caps and mounted on Comfort Hetro Master Shaker for 24 hrs. Soil was allowed to settle, and centrifuged at 3000 rpm for 10 min. Absorbance readings were recorded at 353 nm for oxytetracycline HCl sample solutions and at 270 nm for doxycycline HCl sample solutions using UV-1601 PC, SHIMADZU spectrophotometer.

Polluting Soil with Oxytetracycline HCl and Doxycycline HCl

Seven columns were prepared for the pollution process; each was labeled according to the pollutant type and its quantity. The first column was considered as a blank, i.e, nothing but distilled water was added to it. The second one was polluted with oxytetracycline HCl as a raw material; a solution containing (3.75 g of oxytetracycline HCl/ L) was prepared and added to the column, therefore it was labeled (OTC 1). The third column contained 7.5 g of Oxin 50% powder / L (Oxin 50% contained 500mg/g of oxytetracycline HCl, a product of the Palestinian Company for Veterinary Pharmaceuticals, Ramallah.). The column was labeled (OTC 2). The fourth column contained 15 g of Oxin 50% powder per liter solution, and it was labeled (OTC 3).

The remaining three columns were polluted using doxycycline HCl in the following manner: the first one was polluted with a solution containing (0.75 g/L) of doxycycline HCl raw material and the column was labeled (DOX 1). The second one, was polluted with a solution containing (7.5 g of Doxinal 10% powder /L) added to the column (Doxinal 10% contained 100 mg / g of doxycycline as HCl, a product of Palestinian Company for Veterinary Pharmaceuticals) and labeled as (DOX 2). The last one contained a solution of 15 g of Doxinal 10% powder / L, and the column was labeled (DOX 3). The quantity of the drugs added has been taken from the dosage printed on the label of each product; the dosage was multiplied fifteen times in OTC 1, OTC 2, DOX 1 and DOX 2 and thirty times in OTC3 and DOX 3. The addition of pharmaceutical quantities was based on their dosages printed on labels of each drug. It should be mentioned that all solutions were prepared at the same time and were added, immediately, to the columns after preparation, and doubling doses was a result of frequent use of medications, and the dosages were approved by the Department of Drug Control in the Palestinian Ministry of Health.

Water Addition to Soil Columns

After the addition of pharmaceuticals to the columns, equal amounts of distilled water were added to each column; the addition continued until the emergence of drugs in leachate water from each column. The total amount of distilled water added was 1.8 L to each soil column. Soil columns were left for 24 hrs to ensure a complete adsorption process to soil.

Collecting and Storage of Soil and Leachate Water Samples

Water samples were collected and kept in well- closed HDPE plastic bottles, and stored in a refrigerator (at 7°C). HDPE plastics are known for their low adsorption properties, low moisture absorption, and high tensile strength. HDPE is also non-toxic and non-staining and meets FDA and USDA certification.

Each soil column was divided into three zones (0-20 cm, 20-60 cm and 60-100 cm). Samples of soil were collected from each zone, and kept in well closed HDPE plastic jars for analysis; all jars were stored at room temperature (recorded temperature was 20°C).

Instrumentation

Absorbance readings of both oxytetracycline HCl and doxycycline HCl were detected using UV-VIS HITACHI, model no: U/ 2001. According to the USP 2007, oxytetracycline HCl has an absorbance at 353 nm, and doxycycline HCl has an absorbance at 270 nm, therefore readings of absorbance for both substances were taken at the mentioned wavelengths. Their absorbance wavelengths were confirmed using HPLC (Hitachi, Merk).

Polluted Soil Analysis

Soil samples were classified by region, from which the samples were taken; oxytetracycline HCl and doxycycline HCl absorbance readings were measured from 0-20 cm, 20-60 cm and from 60-100 cm in the blank, OTC 1, OTC 2, OTC 3, DOX 1, DOX 2 and DOX 3 columns. The soil samples were prepared as follows: 20 gm of polluted soil were weighed by Precisa, 205A-SCS, Swiss made electrical balance, transferred in to 250 ml conical flask, 100 ml of distilled water were added and stirred for 30 minutes using Freed Electric magnetic stirrer. The suspension was filtered through Whatman filter papers no. 42, and quartz cells were used during analysis.

Total organic carbon was also studied on the three layers of the blank column, OTC 1, OTC 2, OTC 3, DOX 1, DOX 2 and DOX 3 columns. Using Walkely and Black (1934) test method, the organic carbon in the sample was oxidized with potassium dichromate and sulphuric acid.

The effect of addition of another substance on polluted soil such as magnesium chloride hepta-hydrate ($MgCl_2.7H_2O$) was also studied. Magnesium chloride is used in pharmaceutical formulations. Since magnesium is a chelating metal, it forms complexes with tetracyclines, so the effect of its addition on oxytetracycline and doxycycline concentrations was measured versus time at room temperature. The concentration of the added magnesium chloride hepta-hydrate was 1% on all soil samples. Soil samples were taken from 0-20 cm zone in OTC 2 and DOX 2. Twenty grams of polluted soil were weighed and transferred into 250 ml conical flask and 100 ml of distilled water and 1 gm of $MgCl_2$. 7 gm of H_2O (purchased from Merk, analytical grade) were added and stirred for 30 minutes before every reading. The solution was filtered through Whatman filter paper no. 42, and the absorbance was measured at different time intervals at room temperature.

Polluted Water Analysis

The leachate polluted water was collected and transferred into HDPE plastic bottles, and stored at 7 °C. Polluted water was filtered using Whatman filters no. 42. pH readings were recorded before and after soil pollution. At different time intervals, absorbance readings were recorded at a wavelength of 353 nm for oxytetracycline HCl and at 270 nm for doxycycline HCl. pH readings were recorded before and after pollution for all columns, using Hanna Instruments - pH meter.

Results and Discussion

The results of this work are represented in a graphical and tabular form. Discussion of the results follows each part of the experimental work. Results were devoted to understand the behavior and fate of oxytetracycline HCl and doxycycline HCl as examples of tetracyclines in soil and underground water including their adsorption in soil.

Soil

Samples of soil were analyzed in order to evaluate the soil texture, moisture, organic matter, pH and nitrogen content. Table 1 shows the results obtained for these tests (each result was the average of three readings obtained and all calculations in this chapter were based on dried basis).

Table 2: pH readings for leachate water before & after pollution

Soil Column	Blank	OTC 1	OTC 2	OTC 3	DOX 1	DOX 2	DOX 3
pH before pollution	7.41	7.26	7.38	7.32	7.21	7.33	7.2
pH after pollution	7.41	6.12	6.02	5.92	6.24	6.35	5.96

pH Measurements for the Leachate Water Before and After Pollution

After the washing process with distilled water was done, pH readings for the leachate water were recorded before and after addition of pharmaceuticals in table 2.

From the table, it's obvious that all pH readings were near 7.0, the neutral pH, so that soil pH had neutral effect on the adsorption medium neither acidic nor basic. Except for both pharmaceuticals, oxytetracycline HCl and doxycycline HCl have an acidic character after dissolving in distilled water; therefore, after addition of the pharmaceuticals, soil media was expected to be slightly acidic since both pharmaceuticals used were as the hydrochloride derivative. This could be an indication for an adsorption process occurred while leaching through the soil.

According to other research [4, 5], it was observed that the adsorption of tetracyclines in the native forms of montmorillonite clay decreases with increasing pH in the order pH 1.5 > 5.0 > 8.7 > 11.0. This trend is consistent with cationic exchange interactions that are dominant at lower pH values when tetracyclines have a net positive charge. On the other hand, adsorption of tetracyclines to soil could occur in acidic and basic media, and it is greatly dependent on the pH of soil.

Optimum Time for Oxytetracycline HCl and Doxycycline HCl Adsorption onto Soil

The purpose of this task was to determine the optimum time for the completion of adsorption process of both tetracyclines onto soil to reach equilibrium.

Figure 1 shows concentrations (Conc.) of oxytetracycline and doxycycline solutions after the addition of 50 ml of 0.005% w/v of each of prepared solutions, each with 5 grams of soil sample at different mixing times (1, 2, 4, 6, 12, 24 and 36 hours). Equilibrium occurred after 24 hours of adsorption for both oxytetracycline HCl and doxycycline HCl as shown in figure. 1.

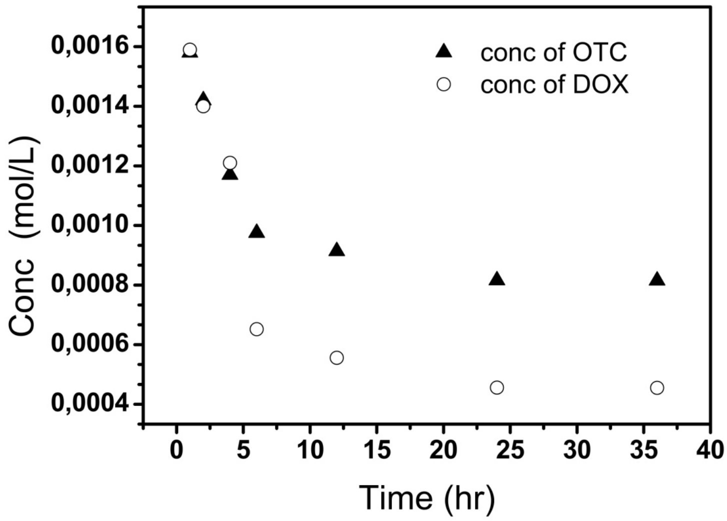

Figure 1: Plot of Concentrations of doxycycline HCl and oxytetracycline HCl vs time.

According to the above results, the optimum time for adsorption was after 24 hours of adsorption, since no change in concentration was noticed in the aqueous layer at that time. Which means equilibrium between the aqueous layer of tetracycline concentration and soil tetracycline concentration is reached after 24 hours, both oxytetracycline and doxycycline adsorptions followed first order kinetics, in both samples 1 and 2 for oxytetracycline and doxycycline readings, by plotting ln(concentration) for each tetracycline vs time, R values (correlation coefficient) of both samples 1 & 2 for oxytetracycline & doxycycline readings were close to 1 .

Adsorption Isotherms

The equilibrium adsorption data could be described by the Freundlich adsorption equation (1):

$$x/m = k\,(C_e)^{1/n} \qquad (1)$$

The Freundlich equation constants "k" & "n" could be obtained form the empirical Freundlich adsorption equation (1):

Where:

x/m: amount adsorbed (mol/g soil); x: mol of compound adsorbed ; m: weight of soil (g)

Ce: equibrium concentration (mol/L); k & n: Freundlich adsorption constants.

The Freundlich constant "k" is related to the extent of adsorption and has been used to correlate adsorption data to various parameters associated with adsorbent, in this case soils, and to various physical parameters e.g. solubility of the adsorbed compound.

Freundlich isotherm constants (k & n) for oxytetracycline HCl & doxycycline HCl & the correlation coefficient "R" were listed in table 3.

Table 3: Freundlich isotherm constants (k & n) & the correlation coefficient R for oxytetracycline HCl & doxycycline HCl

Substance	k	1/n	n	R^2	R
Oxytetracycline HCl	0.841	0.897	1.11	0.969	0.984
Doxycycline HCl	0.728	1.051	0.951	0.999	0.999

However, in many environmental applications, the linear form of the Freundlich isotherm applies. For the linear adsorption isotherm when 1/n = 1. From table 3, n values for both oxytetracycline HCl & doxycycline HCl were found to be close to 1.

The Effect of Organic Matter

Organic matter influences physical and chemical properties of soil often to a critical extent. Organic matter is essential to coarse-grained materials for providing nitrogen and higher cation exchange capacities[6].

Tables 4 and 5, show that the organic matter content in the soil used in this study ranges of between 3.02% - 5.93%, which is considered a moderate organic matter-soil. It also shows the influence of soil organic matter content on adsorption of oxytetracycline HCl and doxycycline HCl in blank, OTC1, OTC2, OTC 3, DOX1, DOX2 and DOX3, which was an evident for all results.

Table 4: Represents concentrations of oxytetracycline HCl in different soil depths compared with organic matter content

Soil Column	Soil Depth	Concentration of oxytetracycline found in mol /Lt	Organic matter
Blank	0 – 20 cm	Non	4.22%
	20 – 60 cm	Non	3.72%
	60 – 100 cm	Non	3.37%
OTC 1 (3.75 gm/Lt)	0 – 20 cm	7.87×10^{-2}	5.26 %
	20 – 60 cm	4.41×10^{-2}	4.70%
	60 – 100 cm	0.01×10^{-2}	3.02%
OTC 2 (7.5 gm/Lt)	0 – 20 cm	0.201×10^{-2}	4.31%
	20 – 60 cm	10.08×10^{-2}	5.36%
	60 – 100 cm	0.01×10^{-2}	3.02%
OTC 3 (15 gm/Lt)	0 – 20 cm	5.201×10^{-2}	4.34 %
	20 – 60 cm	17.08×10^{-2}	5.93 %
	60 – 100 cm	0.02×10^{-2}	3.31 %

Table 5: Represents concentrations of doxycycline HCl in different soil depths compared with organic matter content

Soil Column	Soil Depth	Concentration of doxycycline HCl found in mol /Lt	Organic matter
Blank	0 – 20 cm	Non	4.22%
	20 – 60 cm	Non	3.72%
	60 – 100 cm	Non	3.37%
DOX 1 (0.75 gm/Lt)	0 – 20 cm	23.93×10^{-2}	5.50%
	20 – 60 cm	3.36×10^{-2}	5.102%
	60 – 100 cm	0.02×10^{-2}	4.445%
DOX 2 (7.5 gm/Lt)	0 – 20 cm	8.89×10^{-2}	4.94%
	20 – 60 cm	15.4×10^{-2}	5.348%
	60 – 100 cm	14.41×10^{-2}	5.256%
DOX 3 (15 gm/Lt)	0 – 20 cm	9.91×10^{-2}	4.84 %
	20 – 60 cm	17.1×10^{-2}	5.79 %
	60 – 100 cm	15.22×10^{-2}	5.27 %

Comparison of soil depth and the content of organic matter with concentrations of both tetracyclines measured were calculated from the standard curves of oxytetracycline HCl and doxycycline HCl standard solutions.

From the results of this part of study the role of organic matter content was noticeable; both pharmaceuticals were distributed along the columns. In OTC1 and DOX1 soil columns, the highest concentrations were obtained in area 0-20 cm, where a high percentage of organic matter was detected. In addition, chelation to surface metals could be another factor for the presence of large amounts of tetracyclines on the surface; this was proven in researches[7].

In OTC2 and OTC3 soil columns higher concentrations were found in area 0-20 and 40-60 cm; organic matter content was increased by the presence of drug matrix which contributed in adsorption to soil. Little amount of oxytetracycline was found in area 40-60 in the previous three columns, which was an indication of oxytetracycline low mobility in soil [8].

In DOX2 and DOX3 soil columns, doxycycline concentrations were distributed all over the columns, especially in areas from 40-60 cm and 60-100 cm, indicating a higher mobility of doxycycline than oxytetracycline in soil. On the other hand, doxycycline HCl has higher solubility in water (50mg/ml) than oxytetracycline HCl (50mg/50ml), so it was expected that doxycycline HCl would have higher mobility through soil than oxytetracycline.

Organic matter may be an important sorbent phase in soils and sediments for pharmaceutical compounds that can complex metals by the formation of ternary complexes between organic matter ligand groups and pharmaceutical ligand groups[9].

Several investigations have shown that there is a major adsorption of tetracyclines by reference soil components, such as clays [5] and hydrous oxides of soil [10].

Effect of $MgCl_2.7H_2O$ addition to soil

As known, light, temperature, moisture and duration of storage influence the stability of tetracyclines[11]. In this part of research the effect of magnesium chloride hepta-hydrate ($MgCl_2.7H_2O$) addition on polluted soil was studied. Magnesium ions form complexes with oxytetracycline and doxycycline in the ratio of 1:1 complexes. Absorbance readings were measured at room temperature (recorded 22°C) versus time. All readings were transformed into molar concentrations and were recorded in table 6.

Table 6: Concentrations of doxycycline-Mg complex measured at 270 nm at room temperature

Time (hours)	Concentration (mol/L)	Concentration (mol/L)
0	1.35×10^{-2}	2.70×10^{-1}
24	0.51×10^{-2}	2.70×10^{-1}
48	0.27×10^{-2}	2.70×10^{-1}
72	0.183×10^{-2}	2.70×10^{-1}
216	0.113×10^{-2}	2.70×10^{-1}
348	0.161×10^{-2}	1.31×10^{-1}

In many researches the effect of ionic strength on tetracycline adsorptions was studied, in order to determine the bioavailability of tetracyclines[12, 13] or stability of complexes formed. Adsorption of tetracyclines and metals on soil minerals strongly affects their mobility. In another research the effect of copper II with tetracyclines adsorption on soil was studied; it was found that increasing adsorption of TC (tetracyclines) and Cu(II) on montmorillonite as they coexist in the normal pH environment may thus reduce their mobility[14]. Other research suggested that calcium salts promoted oxytetracycline sorption at alkaline pHs, likely by a surface-bridging mechanism[15]. Magnesium was chosen in this research as a chelating metal; it forms complexes with tetracyclines. Magnesium concentrations in soil are measured in ppm or ppb, but its concentration may be increased due to other factors. In some countries, magnesium is used as de-icer; in this way large quantities of $MgCl_2$ are used to de-ice roads. In addition magnesium chloride is also used in pharmaceutical industry for its chelating ability and its low health risk. As other contaminants it can reach the soil and affect its natural characteristics. For the above mentioned reasons, magnesium chloride was chosen as an example of chelating metal that forms complexes with tetracyclines.

From the results of the previous experiment in section 3.6, the rate of hydrolysis and degradation of doxycycline complex was higher compared to that of oxytetracycline complex during the same period of time. Oxytetracycline showed a tendency to form complexes in which fewer protons were bound than in those with doxycycline. This equilibrium difference between oxytetracycline and doxycycline might be because doxycycline has a better pharmacodynamic effect relative to that of OTC[16].

Polluted Water Analysis

The leachate water that flowed from each soil column was kept in well closed HDPE containers, and stored in refrigerator (at 7°C), all were analyzed by a UV-Vis spectrophotometer. Absorbance readings were recorded, then transformed into concentrations (mol/L) using standard calibration curves, then all were plotted against time. Figure 2 shows a plot of ln [A] versus time, where A is the concentration of tetracycline (mol/L) for every absorbance reading from each column measured at different times. The plots were obtained for both oxytetracycline HCl and doxycycline HCl, which was the indication of first order hydrolysis reaction.

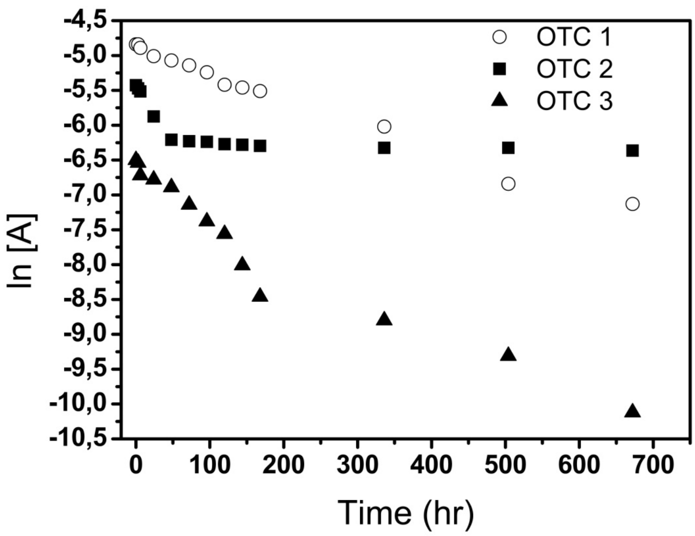

Figure 2: Plot of Ln [A] of OTC1, OTC 2 and OTC3 vs time for polluted water .

In general, hydrolysis rates of tetracyclines increased as pH and temperature, but in this part of experiment pH and temperature were fixed.

As prescribed in figure 2, the concentration measured of oxytetracycline in polluted water was in OTC1 and was the highest amongst the three OTC columns, which was another piece of evidence for the role of organic matter in increasing adsorption onto soil.

On the other hand, doxycycline concentrations in polluted water were found to be highest in DOX3, as shown in figure 3, in spite of the presence of organic matter. However, the effect of hydrophilicity of doxycycline and its high mobility were dominant over the organic matter effect, which was an indication that doxycycline can reach underground water more easily than oxytetracycline can. Although oxytetracycline has low mobility in soil, it can be found in surface and underground water[17]. Also noted, the presence of oxytetracycline in higher concentrations in soil layers is probable to occur due to organic matter effects and chelation with metal ions.

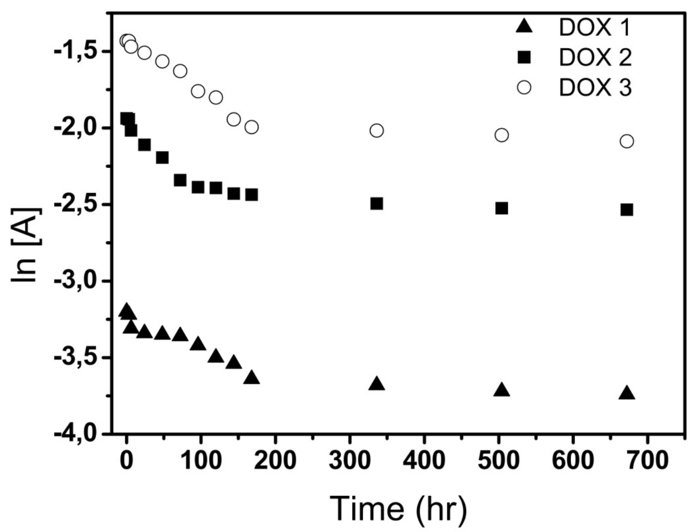

Figure 3: Plot of Ln [A] of DOX1, DOX 2 and DOX 3 vs time for polluted water.

Hydrolysis of oxytetracycline HCl and doxycycline HCl in polluted water followed first order kinetics as prescribed in the plotted graphs as shown in figures 2 and 3.

In other researches, kinetics of tetracyclines degradation follow first order rates[18] and known degradation products were used to confirm that degradation had occurred in polluted underground water [19].

Conclusions

Tetracyclines enter the environment in significant concentrations via repeated fertilizations with liquid manure or via treated animal drinking water, build up persistent residues, and accumulate in soil. Therefore, tetracyclines may have a potential risk, therefore investigations on the environmental effects of these antibiotics are necessary.

The adsorption isotherm curves have the C-type isotherm according to Giles classification and fit to Freundlich isotherms. The values of "n" in Freundlich equation were close to "one", indicating good adsorption for both pharmaceuticals.

Freundlich constant "k", in the Freundlich equation indicates the tendency of a particular compound to be adsorbed on soil particles; the greater the Freundlich constant "k" the greater the adsorption.

The less soluble oxytetracycline HCl has been found to be more adsorptive than doxycycline HCl and thus has higher k value.

pH values of the leachate water before pollution were almost neutral, but after the pollution process was performed, all water samples collected indicated a slightly acidic media, that contributed in better adsorption of tetracyclines on soil.

This laboratory experiment studied several factors that affected the adsorption of tetracyclines on soil; the effect of organic matter was found to contribute to tetracycline adsorption.

Oxytetracycline HCl was more affected by the presence of organic matter than doxycycline HCl. This phenomenon was due to high solubility and high mobility of doxycycline with water. Hydrophobicity and hydrophilicity of organic compounds can be considered another factor of controlling mobility inside soil layers and contributing to pharmaceutical pollution of underground water.

Effect of complex formation may help in sustaining pharmaceutical residues inside soil matrix; this can affect the soil texture, pH and bacterial activity; considering that complexation may reduce the bacterial activity of tetracyclines to some extent.

Oxytetracycline HCl has been found to be more affected by the presence of Mg^{2+} ions in soil forming more stable complexes, than doxycycline HCl.

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