An-Najah National University
Faculty of Graduate Studies

Fate of Oxytetracycline & Doxycycline in Soil & Underground Water

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To My Family…

To My Friends…

To Every One Who Helped Me & Supported Me During My Research…

When you decide change the things, it's because you learn that dreams are only for becoming reality

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After Thanking Allah, who granted me the power to finish this work, I would like to express my deep appreciation to my supervisor Dr. Shehdeh Jodeh for his guidance & support.

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الإقـــــرار

أنا الموقعة أدناه مقدمة الرسالة التي تحمل العنوان:

Fate of Oxytetracycline & Doxycycline in Soil & Under Ground Water

أقر بأن ما اشتملت عليه الرسالة إنما هو نتاج جهدي الخاص، باستثناء ما تمت الإشارة إليه حيثما ورد، وأن هذه الرسالة ككل، أو أي جزء منها من قبل لم يقدم من قبل لنيل أية درجة علمية أو بحثية علمي أو بحثي لدى أية مؤسسة تعليمية أو بحثية أخرى.

Declaration

This work provided in this thesis, unless otherwise referenced, is the researcher's own work, and has not been submitted elsewhere for any other degree or qualification.

Student's name: **اسم الطالبة:**

Signature: **التوقيع:**

Date: **التاريخ:**

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Fate of oxytetracycline & doxycycline in soil & underground water

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Abstract

Pharmaceutical pollution is one of the most serious types of environmental pollution, that attracts increasing attention & lead research studies in recent years. Because of their great impact on aquatic life, soil & under ground water as emerging aquatic micro pollutants that have possibly been affecting the ecological system. It could have major implications on plants, wildlife and humans who may be directly & indirectly be responsible of this type of pollution. In this study two antibacterials were selected, oxytetracycline & doxycycline as examples of pharmaceuticals that are released into the environment, both are marketed in the Palestinian market either for 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, in addition their effect on characteristics of under ground water, all were studied using the UV-Vis spectrophotometry. The results showed that increasing organic matter increases the adsorption of oxytetracycline more than doxycycline, also showed that the composition of oxytetracycline complex with magnesium ion was more stable than doxycycline complex with magnesium. The study also revealed a higher concentration of doxycycline in leachate water from the soil than those of oxytetracycline, because doxycycline has higher solubility in water. It also showed a decrease of the concentrations for both substances over time in

leachate water due to degradation. The degradation of both pharmaceuticals in soil & water would be produced by other substances may be harmful, as the threat of their presence in the soil and groundwater would increase the resistance of bacteria in the soil, in another words that would affect the natural properties of soil and groundwater as well.

Chapter One
INTRODUCTION

Pharmaceuticals are becoming an emerging environmental issue that attracts increasing attention in recent years, as emerging aquatic micro pollutants that 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, and prescription drugs such as hormones, corticosteroids & 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", it could have major implications on wildlife, agriculture, humans & 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]. When people take medication, only a fraction 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 test for pharmaceuticals [3].

The disposal of unwanted or expired drugs is another way that pharmaceuticals enter the wastewater stream. When people dispose of medications, it is common to pour them down the sink or flush them down the toilet. It is also common for people to dispose of pharmaceuticals by throwing them in the trash, in which case they end up in landfills and may eventually enter waterways through leachate [4,5,6].

Some other typical reasons for the disposal of medications are that the medication has expired or it's no more needed because the problem is solved, or house cleaning for the stored medications, & the means of exposal are sink, toilet & trash [7].

Many researches were made on the basis of evaluating the quantity of disposed medications; others were based on pharmaceutical type [8,9,10].

Among various kinds of pharmaceuticals, antibiotics were more frequently detected than others. They are difficult to be removed through common biological treatment methods. Meanwhile, they may adversely affect key biotransformation processes of other pollutants (gentrification, nitrogen fixation, degradation of organic compounds, etc.) [8,9,10].

According to recent research, variety of antibiotics were detected in various water samples including hospital wastewater, municipal wastewater, effluent of wastewater treatment plant, antibiotics industry

wastewater, livestock farm mud and wastewater, surface water, underground water and drinking water [7,11].

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 & DOX on pH of leachate water before & after pollution & their concentrations in leachate water were measured versus time. Oxytetracycline & Doxycycline were chosen because of their wide application here in Palestine, high-solubility in water and high residual toxicity.

Locally, both OTC & 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 & DOX belong to tetracycline antibiotics that are indicated to treat infections caused by gram positive & gram negative bacteria. According to Palestinian Ministry of Health tons of antibiotics are consumed every year, the average of their consumption in both humanitarian & veterinary sector reached 2.6 tons of doxycycline HCl & 4.5 tons of oxytetracycline HCl in the last two years.

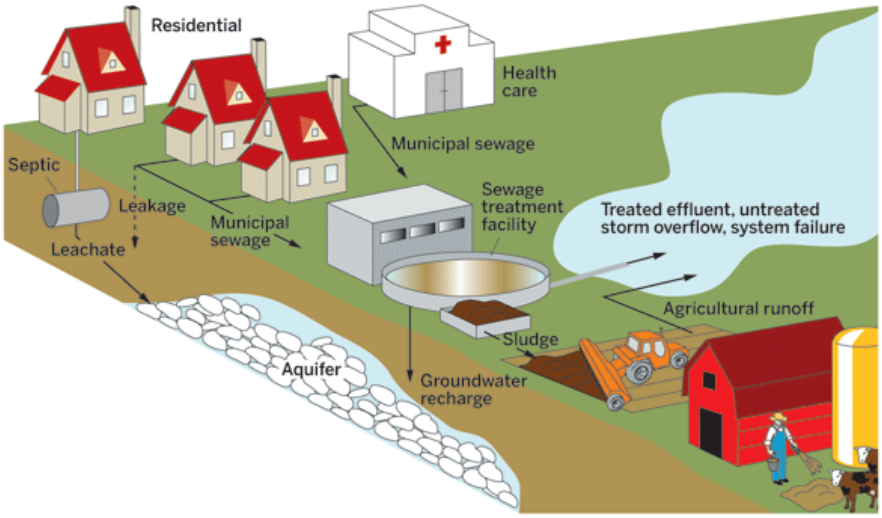


Fig (1.1): Drug flow Pharmaceuticals and their metabolites enter municipal sewage systems and aquifers from homes, health care facilities, and farms [12].

Chapter Two
Literature Review

2.1 Tetracyclines

2.1.1 Definition & Uses

Tetracyclines are a group of broad-spectrum antibiotics whose general usefulness has been reduced with the onset of bacterial resistance. Despite this, they remain the treatment of choice for some specific indications [13].

They are so named for their four (“tetra-”) hydrocarbon rings (“-cycl-”) derivation (“-ine”). More specifically, they are defined as "a subclass of polyketides having an octahydrotetracene-2-carboxamide skeleton" [14]. They are collectively known as "derivatives of polycyclic naphthacene carboxamide".

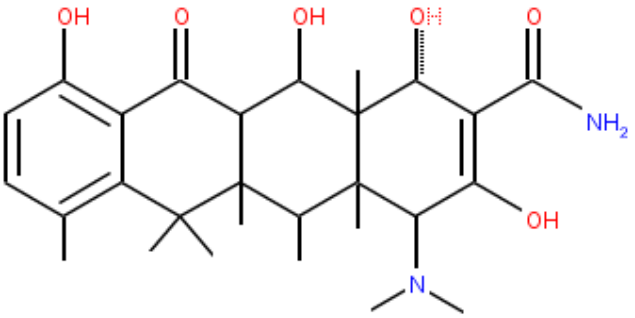


Fig. (2.1): The four rings of the basic tetracycline structure [14]

Tetracyclines are antibacterials used in pharmaceutical industry & veterinary drugs. They are indicated in the treatment of infections caused by gram positive and gram negative bacteria, such as respiratory tract infections, urinary tract infections, Brucellosis caused by Brucella species, Relapsing fever caused by Borrelia sp, Infections caused by Chlamydia

trachomatis such as uncomplicated urethral, endocervical, or rectal infections, inclusion conjunctivitis, trachoma and lymphogranuloma venereum, Tularemia caused by Francisella tularensis, Plaque caused by Yersinia pestis, Cholera caused by Vibrio cholera[15].

Veterinary indications includes treatment of respiratory infections: bronchopneumonia, shipping fever (pasterellosis), atrophic rhinitis & enzootic pneumonia in pigs, mixed infections & necrobacilliosis, gastro intestinal infections caused by E.coli, salmonella & anaerobes, urinary infections, (endo) metritis, acute mastitis, septicemia, infectious polyarthritis, leptospirosis, foot rot, erysipelas, infected wounds , skin infections (exudative epidermitis in piglets), bacterial infections secondary to viral ones, anaplasmosis & heart water [16].

2.1.2 Historical Back Ground

The first member of the group to be discovered was chlortetracycline (aureomycin) in the late 1940s by Dr. Benjamin Duggar, a scientist employed by Lederle Laboratories who derived the substance from a golden-colored, fungus-like, soil-dwelling bacterium named Streptomyces aureofaciens. Oxytetracycline (Terramycin) was discovered shortly afterwards by A.C. Finlay et al., it came from a similar soil bacterium named Streptomyces rimosus. Robert Burns Woodward determined the structure of oxytetracycline enabling Lloyd H. Conover to successfully produce tetracycline itself as a synthetic product [14]. The development of many chemically altered antibiotics formed this group. In June 2005,

tigecycline, the first member of a new subgroup of tetracyclines named glycylcyclines was introduced to treat infections which are resistant to other antimicrobics including conventional tetracyclines [17]. Doxycycline is a member of the tetracycline antibiotics group and is commonly used to treat a variety of infections. Doxycycline is a semi-synthetic tetracycline invented and clinically developed in the early 1960s by Pfizer Inc. [18,19]

2.1.3 Pharmacokinetics

Most tetracyclines are only partially absorbed from the alimentary tract, enough remaining in the intestine to alter the flora & cause diarrhea. They are distributed throughout the body & cross the placenta. Tetracyclines are excreted mainly unchanged in the urine & should be avoided with renal function is severely impaired. Exceptionally among the tetracyclines, doxycycline & minocycline are eliminated by nonrenal routes & may be used in patients with impaired renal function because of this property [20].

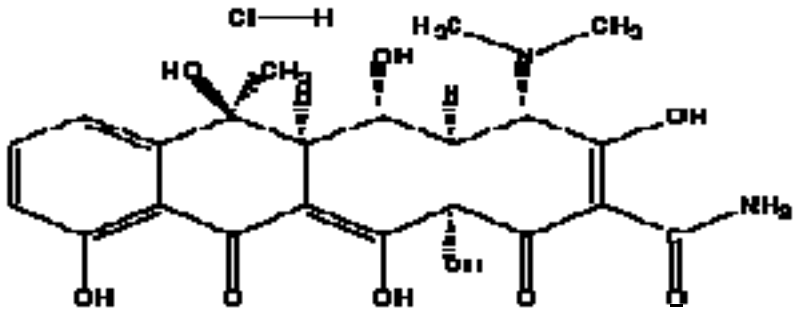
2.2 Oxytetracycline

Oxytetracycline was the second of the broad-spectrum tetracycline group of antibiotics to be discovered. It is also called tetracycline. It is used to treat bacterial infections [15].

2.2.1 History

It was first found near Pfizer laboratories in a soil sample yielding the soil actinomycete, streptomyces rimosus by Finlay et al. In 1950, a

celebrated Scottish American biochemist, Robert B Woodward, worked out the chemical structure of oxytetracycline, enabling Pfizer to mass produce the drug under the trade name, terramycin. This discovery by Woodward was a major advancement in Tetracycline research and paved the way for the discovery of an Oxytetracycline derivative, Doxycycline which is one of the most popularly used antibiotics today [14].



IUPAC Name: (4S,4aR,5S,5aR,6S,12aS)-4-(dimethylamino)-3,5,6,10,11,12a-hexahydroxy-6-methyl-1,12-dioxo-1,4,4a,5,5a,6,12,12a-octahydrotetracene-2-carboxamide hydrochloride

Fig. (2.2): Chemical structure of Oxytetracycline Hydrochloride [14]

2.2.2 Mode Of Action

Oxytetracycline is supplemented as oxytetracycline hydrochloride in most medications; it works by interfering with the ability of bacteria to produce proteins that are essential to them. Without these proteins the bacteria cannot grow, multiply and increase in numbers. Oxytetracycline therefore stops the spread of the infection and the remaining bacteria are killed by the immune system or eventually die. Some strains of bacteria have developed resistance to this antibiotic, which has reduced its effectiveness for treating some types of infection. [4]

2.2.3 Indications

Oxytetracycline is still used to treat infections caused by Chlamydia (e.g the chest infection psittacosis, the eye infection trachoma, and the genital infection urethritis) and infections caused by mycoplasma organisms (eg. pneumonia) [4].

Oxytetracycline is used to treat acne, due to its activity against the bacteria on the skin that cause acne (Propionebacterium acnes). It is used to treat flare-ups of chronic bronchitis, due to its activity against the bacteria usually responsible, Haemophilus influenza [4].

Oxytetracycline may also used to treat other rarer infections, such as those caused by a group of micro-organisms called rickettsiae (eg Q fever). To make sure the bacteria causing an infection are susceptible to oxytetracycline the doctor usually takes a tissue sample, for example a swab from the infected area, or a urine or blood sample [4].

2.2.4 Veterinary Indications

Oxytetracycline is indicated in the treatment of respiratory infections: bronchopneumonia, shipping fever (pasterellosis), atrophic rhinitis & enzootic pneumonia in pigs, mixed infections & necrobacilliosis. Gastro intestinal infections caused by E.coli, salmonella & anaerobes. Urinary infections, (endo) metritis, acute mastitis. septicemia, infectious polyarthritis, leptospirosis, foot rot, erysipelas, infected wounds & skin

infections (exudative epidermitis in piglets). Bacterial infections secondary to viral ones. Anaplasmosis, heart water [14].

2.2.5 Contra Indications

Oxytetracycline is not indicated in cases of renal & hepatic insufficiency, but if necessary, in such cases the dosage levels may be reduced, also in cases of hypersensitivity to tetracyclines. [17]

2.2.6 Side Effects

Heartburn, nausea & vomiting due to gastric irritation are common. Tetracyclines including Oxytetracycline (OTC) are selectively taken up in the teeth and growing bones of the fetus and of the children. Due to their chelating properties with calcium phosphate. Bacterial resistance may develop after long term use of antibiotics [17].

2.2.7 Interactions

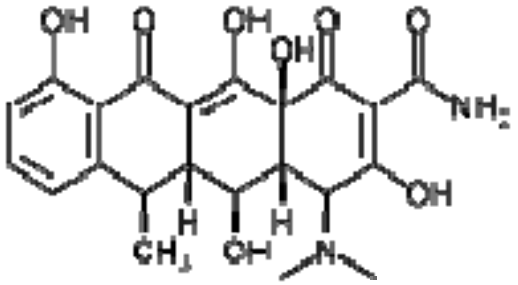
Avoid using or diluting oxytetracycline with aluminum, magnesium & calcium containing solutions or with penicillins & cephalosporins. Using tetracyclines with aminoglycosides may decrease the bactericidal activity of aminoglycosides. [17]

2.3 Doxycycline

Doxycycline is a member of the tetracycline antibiotics group and is commonly used to treat a variety of infections & for veterinary uses. It is a semi synthetic tetracycline. [15]

2.3.1 History

Doxycycline was developed in the early 1960s by Pfizer Inc. and marketed under the brand name vibramycin. Vibramycin received FDA approval in 1967, becoming Pfizer's first once-a-day broad-spectrum antibiotic [15].



IUPAC Name: (4S,4aR,5S,5aR,6R,12aS)-4-(dimethylamino)-3,5,10,12,12a-pentahydroxy-6-methyl-1,11-dioxo-1,4,4a,5,5a,6,11,12a-octahydrotetracene-2-carboxamide

Fig. (2.3): Chemical structure of Doxycycline [14]

2.3.2 Mode of Action

Doxycycline is supplemented as doxycycline hydrochloride or hyclate (mono hydrate complex with ethanol) in most medications. Like other tetracyclines, doxycycline works out by blocking protein synthesis & preventing the binding of aminoacyl-tRNA to the ribosome. Its action is bacteriostatic (preventing the growth of bacteria) rather than killing (bactericidal). [21]

2.3.3 Indications

Doxycycline is effective against a broad range of Gram-positive and Gram-negative bacteria and Rickettsia. It is indicated in the treatment of

inflammatory diseases & Lyme disease,[22-25] ehrlichiosis [26-27] and Rocky Mountain spotted fever. In fact, because doxycycline is one of the few medications shown to be effective in treating Rocky Mountain spotted fever (with the next best alternative being chloramphenicol), doxycycline is indicated even for use in children for this illness. Otherwise, doxycycline is not indicated for use in children under the age of 8 years. Doxycycline, like other antibiotics, will not work for colds, flu, or other viral infections. Doxycycline may also be used to treat and prevent Escherichia coli, spotted fever, folliculitis, acne, shigella species, respiratory tract infections caused by haemophilus influenzae, respiratory tract and urinary tract infections, Upper respiratory infections caused by streptococcus pneumoniae, & in cases of malaria prophylaxis. [14]

2.3.4 Veterinary Indications

Doxycycline is also indicated for veterinary use against infections caused by gram positive & gram-negative bacteria & against anaplasma in poultry, turkey & cage birds. It is also indicated in the treatment of respiratory tract ornithosis & psittacosis ocular infections caused by chlamydia psittaci or mycoplasma [16].

2.3.5 Contra Indications

Doxycycline is contraindicated in cases of pregnancy, especially during the last half of pregnancy period or to children under the age of eight. It is also not indicated in cases of sensitivity to tetracylines [14, 16].

2.3.6 Side Effects

Cautions and side effects are similar to other members of the tetracycline antibiotic group. However the 10% risk of photosensitivity skin reactions is of particular importance for those intending long-term use for malaria prophylaxis because it can cause permanent sensitive and thin skin [13] .Long treatment with Doxycycline HCl may affect the growth of intestinal bacteria. & may also cause vomiting & diarrhea [16,21].

2.3.7 Interactions

Doxycycline is not to be administered with aluminum, calcium, magnesium containing compounds [15], or with iron containing compounds or with anti-diarrheal compounds containing kaolin & pectin or bismuth & laxatives, since it reduces the G.I.T absorption of doxycycline, or drug with sodium bicarbonate since it affects its absorptivity, or with the antibiotics (Penicillin, cephalosporin & aminoglycosides) because it may decrease the bactericidal activity of them [16]. Doxycycline may interact with anticoagulants and its effectiveness is lowered by over the counter antacids and bismuth subsalicylate, barbiturates, the anticonvulsants carbamazepine and phenytoin [21].

In addition, if it is used in conjunction with the anesthetic methoxyflurane there can be severe or fatal kidney damage [21].

2.4 Antibiotics in Animal Husbandry

Antibiotics have been used in animal husbandry for more than half a century now. They are administered to all species of food animals, including fish, for three types of use:

i) Therapeutic use is aimed at curing infected animals. The substances are administered through injection, feed or water. If groups of animals are treated, it may be that some are not diseased or are in a subclinical stage of the disease.

ii) Prophylactic use is aimed at preventing a disease. The substances are typically administered through feed to groups of animals. Although not diseased yet, some of the animals may be subclinical or can be expected to become infected. This is likely in situations when animals are moved to different environments with different pathogens, as e.g. from breeding to fattening units.

iii) Subtherapeutic use is aimed at growth promotion or increased feed efficiency. As in prophylactic use, the substances are administered through feed, but at lower doses. While neither an actual nor an expected disease is the indication for this type of use, it may have the side effect that diseases are prevented, i.e. become less likely [28].

The use of antibiotics in animal husbandry is associated with a number of benefits and risks, which are described in brief in the next subsection.

Benefits and Risks

There are three areas in which benefits of the application of antibiotics in animal husbandry can be identified: food safety and quality, costs and efficiency, and environmental effects.

i) Improved food safety and quality can be observed due to healthier animals in general and thus due to reduced pathogen contaminations of animal products [29].

ii) Cost reduction due to lower loss rates and productivity gains, e.g. enhanced growth in fattening animals [30].

iii) As a consequence of the above mentioned increases in productivity and feed efficiency, antibiotics contribute to reduced emissions of nitrogen, phosphorus and methane per unit of output [31, 32].

Although critical voices have been raised since the very beginning of antibiotic use in agriculture, it has only been in the past decade that the risks of antibiotics have received considerable public attention [33].

Possible hazards relate to the furthering of zoonotic pathogens, which can spread from animals to humans and thus pose a threat to consumers who may get infected from contaminated food. Well known bacteria of that type are salmonella, listeria and campylobacter. Other possible hazards are toxicity and allergenicity of antibiotic substances, i.e. residues in food as a food safety issue, and development of antibiotic-resistant pathogens in humans and animals [28, 33, 34].

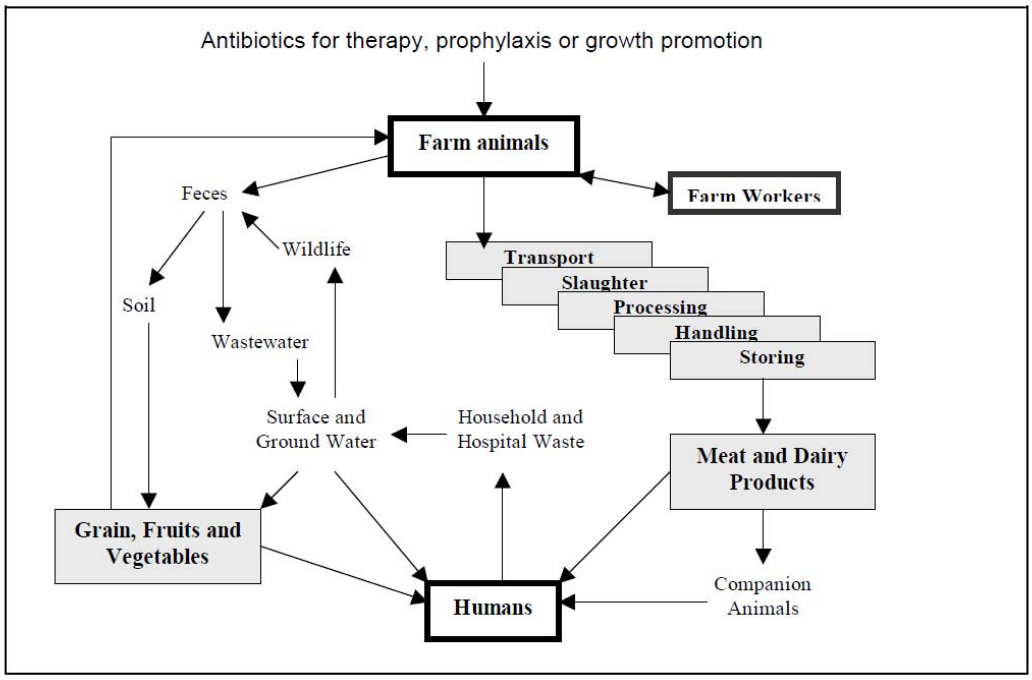


Fig. (2.4): The flow of resistance from bacteria in farm animals to humans [32]

2.5 Antibiotics in the Aquatic & Terrestrial Environment

People all over the world prescribes millions of doses of prescription drugs, Livestock are given millions more. But after the pill has been swallowed or the injection taken, the active components of the drugs do not become inert or completely absorbed by the body. After the excretion of drugs from the body, pharmaceuticals start to appear again in waste water, soil & under ground water.

In a statistical research, it was found that humans consume 235 million doses of antibiotics each year, livestock & poultry producers administered more than 21 million pounds of antibiotics to animals in the year 2004 alone [35].

Current estimates are still being gathered, but a study conducted in 1999-2000 by the US geological survey (USGS) found that most waterways contain at least some antibiotics, steroids, synthetic hormones or other common drugs. Out of 139 streams in 30 states, it was found that about 80% contained trace amounts of contaminants, half of the streams contained seven or more chemical compounds, one third of the streams contained 10 or more compounds & one water sample contained 38 chemicals [36].

2.5.1 Tetracyclines in Soil

Sorption of tetracyclines in soil was reported in many studies, Tetracycline Residues in Soil Fertilized with Liquid Manure by High-Performance Liquid Chromatography with Electrospray Ionization Tandem Mass Spectrometry [37], A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment [38], Column studies to investigate the fate of veterinary antibiotics in clay soils following slurry application to agricultural land [39], Sorption of Tetracycline and Chlortetracycline on K- and Ca-Saturated Soil Clays, Humic Substances, and Clay−Humic Complexes [40], Adsorption characteristics of tetracycline by two soils: assessing role of soil organic matter [41].

2.5.2 Tetracyclines in Under Ground Water

Tetracyclines were detected in environmental water samples using various analytical techniques, such as liquid chromatography with

fluorometric detection & solid phase extraction [42], other studies focused on the study of behavior of tetracyclines in the presence of sulfonamides and measured their adsorption coefficients in soil & leaching to the underground water [43], the study shows that tetracyclines and sulfonamides show distinctly different environmental behaviors. One explanation may be their different sorption coefficients in soil, indicating (in part) their different mobilities in the ecosystem. Tetracycline resistance genes were also has been detected in the underground water, in which it was an indication of the presence of tetracyclines in the underground water [44].

2.5.3 Sources of Antibiotics in the Environment

In spite of all of the benefits of having a healthy microbial population, antibiotics and antibacterial agents are added to the environment at a rate of over a million pounds per week. There are several routes of entry of antimicrobial agents into the environment. Studies have shown that introduction by these routes has changed the antibiotic susceptibility of the microbes in those environments and/or changed the predominant microbes.

- Sewage. The antibiotics that we take in are not all processed by our bodies. Some of them are expelled as waste and wind up in our waste water treatment plants. Of bacteria isolated from sludge remaining after wastewater treatment at one plant, 46.4% were resistant to multiple antibiotics. Sewage from hospitals and pharmaceutical plants has been shown to contribute to antibiotic resistance in treatment plants. Rivers

contaminated with urban effluent and agricultural runoff have also been shown to have greater antibiotic resistant bacterial populations than areas upstream of the contamination source. Antibiotic resistance in streams is also indirectly selected for by an increase in industrial wastes containing heavy metals.

- Medical waste. The dispensing of antibiotics in a medical facility inevitably leads to waste. Discharge from hospitals has been shown to cause an increase in bacterial populations resistant to certain antibiotics such as oxytetracycline.

Microbes are becoming resistant to antibiotics due to environmental pollution, overuse of antibiotics, and antibacterial agents.

- Production. Antibiotic sales total more than $8 billion worldwide each year. That is 50 million pounds produced each year, 25 million pounds of which are prescribed for human use. Discharge of wastewater from pharmaceutical plant has been associated with an increase in the prevalence of single- and multiple-antibiotic resistance in indicator organisms.

- Household products. Over 700 “antibacterial” household products have been introduced in the past five years. These include such items as sweat socks, toothpastes, kitchen plastics, cement and paints. The more common antibacterial ingredients in these formulations are triclosan, quartenary ammonium compounds, alcohol, and bleach. Microbes

resistant to each of these compounds have been documented in nature and in some human pathogens. These products wind up in the sewage or landfill after being used in our households.

- Sprayed on crops. About 300,000 pounds of antibiotics are used in plant production each year. They are sprayed on high-value crops such as fruit trees to prevent bacterial infections. This can select for resistant bacteria on crops. Not all of the spray remains on the fruit. Most of the antibiotics are washed into the soil and eventually end up in the ground water.

- Animal production. Antibiotics are commonly added at subtherapeutic levels to animal feeds as growth promoters. They are also added to fishery waters. About 24 million pounds of antibiotics are fed to animals every year. Due to this practice antibiotic resistance in foods has become a health concern. Bacteria such as drug resistant Salmonella typhimurium, Escherichia coli and Enterococcus have increased clinically as animal antibiotic use has risen. It is also possible that our normal gut microbiota have gained antibiotic resistance from antibiotic-exposed food animals. A popular theory is that vancomycin resistant strains of the bacterium Enterococcus (VRE), a major cause of postsurgical infections, have arisen in Europe due to the use of the antibiotic avoparcin as an animal growth promoter. At least one study, however, shows that in minced beef and pork, VRE occurs very rarely. The use of oxytetracycline in aquaculture has been shown to cause a

seasonal shift in bacterial species towards Enterobacteriaceae and is associated with increased antibiotic resistance [45].

2.5.4 Bacterial Resistance on the Rise

The World Health Organization (WHO) has recently identified antibiotic resistance as a major problem for public health on a global scale. While overuse and inconsistent application in human medicine have been found to be probably the most important sources of risk, the use of antibiotics in animal husbandry may also contribute to the problem. However, both the complexity of the issue and the lack of data prove to be serious obstacles on the way to evaluating the possible risks from that source of resistance [32, 33]. There has been worries about the massive amounts of antibiotics used to treat livestock may be creating antibiotic-resistant microbes. "There's a whole other source of pharmaceutical pollution that really needs attention, and that's livestock use, which generates an estimated 500 million tons of waste each year," says Dana W. Kolpin, a research hydrologist at U.S. Geological Survey (USGS) who studies emerging contaminants in the environment. Kolpin points out that livestock manure is full of antibiotics, synthetic and biogenic hormones, and other veterinary medicines. Farmers use sludge generated by sewage treatment plants as a fertilizer and a source of nutrients for crops, but this material also contains excreted medications [12]. Experts claim that bacterial resistance will make possible infectious disease epidemics more potent and deadly than any have been experienced in human history.

However, with global travel and widespread commerce, drug resistance can be expected to spread steadily to all parts of the world. Developing countries might thus suffer the worst consequences because of the poor state of their health services and their inability to pay for alternatives to cheap antibiotics. Pharmaceutical companies should see that it is in their own interest to minimize drug use and pollution of the environment, since avoiding the spread of resistance will keep their medicines effective longer [46, 47].

It was reported that 150 genes are known to be responsible for the development of resistance, which may occur in seven different modes or strategies. Furthermore, resistance capabilities do not remain contained within the bacteria population where they were developed. They may not only be inherited, but can also be transferred to other bacteria through so called plasmides which have stored the genetic information on one or more resistance. This transfer is not restricted to organisms of the same species but may also happen between different bacterial species. This process is the cause of cross resistance, which may occur both within and between pathogen and non pathogen strains, which might also serve as resistance reservoir for pathogens. Antibiotics affect the spreading of resistance by heritage or transfer through the selective pressure they exert on bacterial populations. The presence of an antibiotic substance alters the environment in favour of those bacteria that are resistant to it [48]. Figure 2.4 describes the direct proportion between the use of antibiotics & the formation of resistance strains.

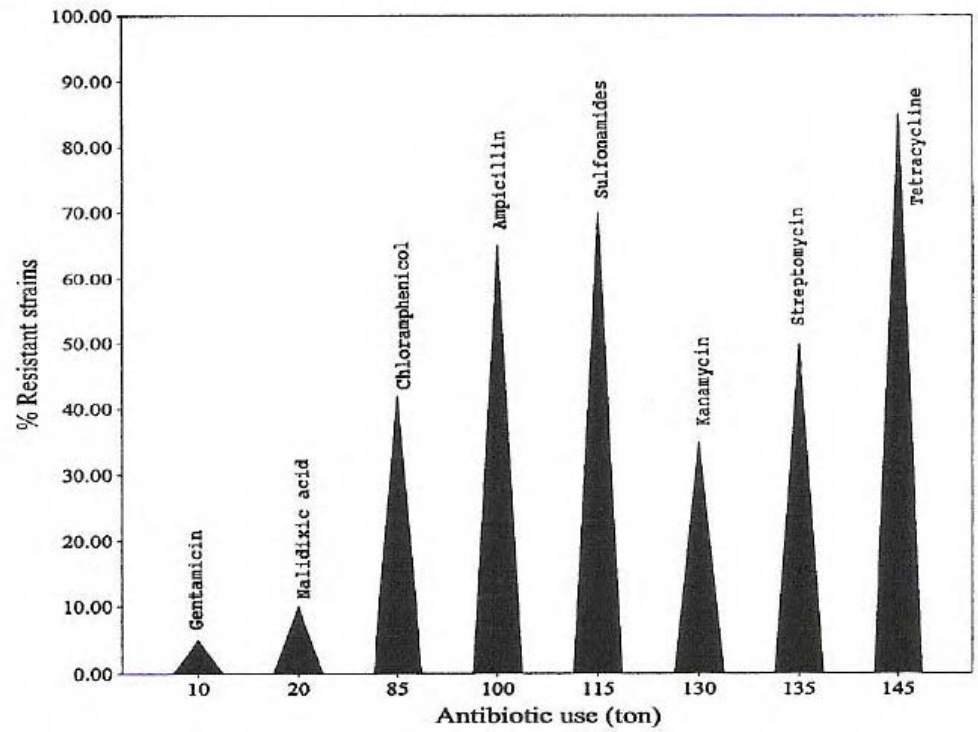


Fig. (2.5): The relationship between antibiotic use and increase in antibacterial resistance [49]

2.6 Adsorption onto Soil

2.6.1 Adsorption Process

Adsorption is a surface phenomenon that is defined as the increase in concentration of substance at an interface between two phases which can be solid-liquid. Adsorption from solution onto solid occurs as a result of one two characteristic properties for a given solvent-solute-solid system. The primary driving force for adsorption may be consequence of (solvent disliking) character of the solute to the solvent, or high affinity of the solute for the solid [50]. The second primary driving force for adsorption results from a specific affinity of the solute for the solid, where the atoms at the solid's surface are subjected to unbalanced forces of attraction which are

normal to surface plane and are merely extension of the forces acting within the body of the material and ultimately responsible for the phenomenon of adsorption [51]. The adsorption process includes electrical attraction of the solute to adsorbent, Van- der Waals attraction or of chemical nature.

Physical adsorption dose not involve sharing or transferring of electrons & maintains the individuality of interacting species. The interactions are fully reversible where adsorption occurs at the same temperature and the process may be slow because of diffusion effects, but chemical adsorption involves chemical bonding and is irreversible. In physical adsorption molecules are free to undergo translation movement within the interface, but in chemical adsorption, molecules are considered not to be free to move on the surface where they are attached to active centers. Therefore in chemical adsorption molecules being saturated when each active center is occupied and adsorption dose not exceed beyond the first layer, but it is possible that additional physical adsorption occurs [52]. The heat of physical adsorption is low compared to that of chemical adsorption & chemical interaction between adsorbent & adsorbate is favored by high temperature.

Most adsorption phenomena are combination of the three adsorption forms that is the several forces often interact to cause concentration of particular solute at an interface [53].

2.6.2 Adsorption Equilibrium Isotherms

Adsorption equilibrium is a physic-chemical aspect which determines the ultimate adsorption capacity. As the adsorption process proceeds, the adsorbed solute tends to desorbs into solution. Ultimately equal amounts of solute are absorbed & desorbed simultaneously, where no change can be observed in the solute concentration. Consequently the adsorption process attains equilibrium state called adsorption equilibrium. The equilibrium position is characteristic of the entire system, the solute, adsorbent, solvent, temperature, pH, and so on [53]. At this equilibrium position, there is a defined distribution of solute between the solid and the liquid phases, also the adsorbed quantity usually increase of solute concentration.

The presentation of the amount of solute adsorbed per unit of adsorbent as a function of the equilibrium concentration of adsorbent in the bulk solution under a set of experimental conditions is termed as the adsorption isotherm.

The shape of an isotherm gives qualitative information about the adsorption process. These can be broken down into four main classes, each of which can include several relatively minor variations [54, 55], fig 2.6 shows Giles isotherm classifications.

1- **S class**: of the four main types, it is the most difficult to explain fully, since the shape appears to depend upon the interaction of a number of

factors. It is indicative of vertical orientation of adsorbed molecules at the surface.

2- **L class**: the normal isotherm, it may be said to occur with majority of system. Usually indicative of molecules adsorbed flat on the surface of the solid or vertically oriented adsorbed ions with particularly strong inter molecular attraction or adsorption of ionic micelles.
3- **H class**: high affinity, this is a special case of the previous class caused by very high solute affinity (high affinity ion exchange with low-affinity ions) and produced by adsorption of large units or chemical adsorption.
4- **C class**: constant partition, linear curves, where the solute penetrates the solid more readily than the solvent & tends to open up the structure. In other words, the solute rather than solvent initiates adsorption.

Several types of isotherms relations may occur. The most common relationship is in which adsorption from solution leads to the deposition of apparent single layer of solute molecules on the solid surface. Occasionally multi molecular layers of solute may be adsorbed. The Langmuir & Freundlich isotherms are valid for single layer, where as Brunauer Emmett & Teller (BET) isotherm represents multilayer adsorption. Both Langmiur & BET equations are limited by the assumption of unifom energies of adsorption on the surface [51].

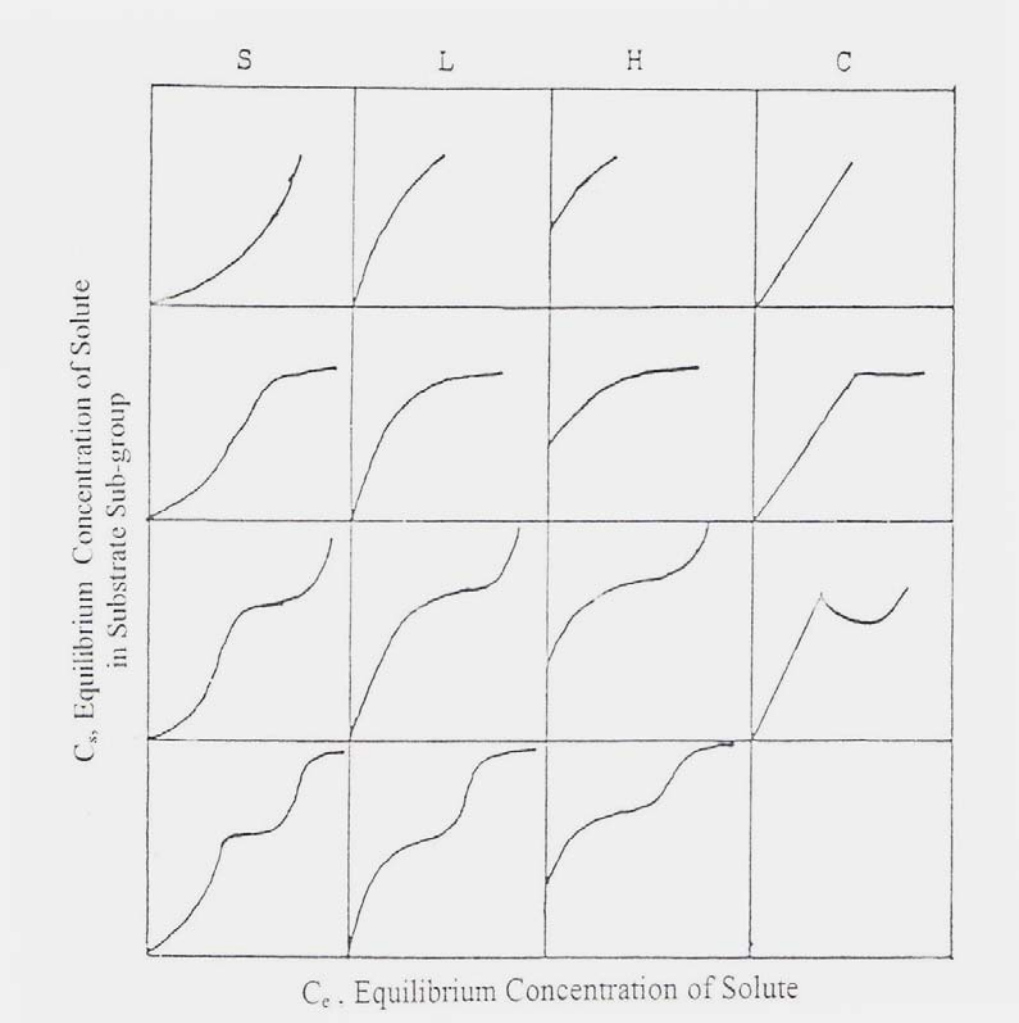


Fig. (2.6): Giles isotherm classification [54]

The Langmuir equation is expressed as [56]:

$$\frac{X}{m} = \frac{X_m\, bC_e}{1 + bC_e} \qquad (2.1)$$

Where:

m: weight of adsorbent (mg, g)

C_e: equilibrium concentration of the solute.

X_m: amount of solute adsorbed per unit weight of adsorbent required for monolayer coverage of the surface. (maximum capacity for monolayer capacity).

b: a constant related to the heat of adsorption, 1/unit weight.

For linearization of the equation (2.1), it can be written in the form:

$$\frac{C_e}{x/m} = \frac{1}{bx_m} + \frac{C_e}{x_m} \qquad (2.2)$$

or

$$\frac{1}{x/m} = \frac{1}{x_m} + \left(\frac{1}{bx_m}\right) \cdot \left(\frac{1}{C_e}\right) \qquad (2.3)$$

Any of these equations may be used to evaluate b & x_m from experimental data using graphic or linear least squares analysis [57].

Freundlich adsorption equation is perhaps the most widely used mathematical description of adsorption in aqueous systems. The Freundlich equation is expressed as [58]:

$$\frac{x}{m} = KC_e^{1/n} \qquad (2.4)$$

Where

x: amount of solute adsorbed (mg, mole)

x_m: weight of adsorbent (mg, g)

C_e: equilibrium concentration of solute

K: constant, a measure of adsorption capacity

(1/n): constant, a measure of adsorption intensity

It is generally stated by Helby (1952) that values of n in the range 2-10 represent good adsorptions [58]. Estimation of these constants is possible by simple transformation of equation (2.4) to logarithmic form:

$$\log x/m = \log k + 1/n \log C_e \qquad (2.5)$$

Plotting log x/m versus log C_e a straight line is obtained with a slope of 1/n, and log k is the intercept.

Although the Freundlich equation has no theoretical basis, it has been found to be more adaptable to the adsorption data than the theoretically derived Langmuir equation; this is due to the fact that the majority of adsorption processes do not comply the Langmuir equation assumption of existence of monolayer in the adsorption of solute solution [60].

The Brunauer-Emmett-Teller (BET) equation is commonly written as shown in equation 2.6[53]:

$$\frac{x}{m} = \frac{x_m B C_e}{(C_s - C_e)\,[1 + (B - 1)C_e/C_s]} \qquad (2.6)$$

Where x, m, x_m & C_e have the same meaning as Langmuir's isotherm. B is a constant describing the energy of interaction between the solute & the adsorbent surface, and Cs is the solubility of solute in water at a specified temperature. The transformation of equation (2.6), shows that a plot of the left side against C_e/C_s should give a straight line having slope $(B-1)/x_mB$ & intercept $1/x_mB$:

$$\frac{C_e}{x(C_s - C_e)} = \frac{1}{x_m B} + \frac{(B - 1)}{x_m B}\,\frac{C_e}{C_s} \qquad (2.7)$$

The adsorption isotherms are useful in predicting the amount of adsorbent needed in a batch process for producing a desired residual solute level.

Chapter Three
Research Methodology

3.1 Experimental Work

The experimental work in this research depended basically on determining the concentration of residues of oxytetracycline HCl & doxycycline HCl versus time in soil & leachate water (in which it was considered here as the underground water) after adsorption for 24 hours. Samples of soil & 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 also. 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 were cleaned & dried before each measurement. Standard readings were obtained for oxytetracycline HCl & doxycycline HCl and plotted against absorbance readings, in order to calculate the concentrations of both substances in soil & leachate water.

3.2 Materials & Methods

Soil Column Preparation

In this study seven soil columns were prepared from PVC plastic, the dimensions were 1 meter long & 6 inches in diameter, the soil was gathered from 600 m^2 area located on the top of Mount Gerizim in Nablus city, far away from any expected source of contamination with any pharmaceuticals type. Randomly, and from different sites, the soil was collected, mixed &

filled inside the columns; two kilograms were taken & sieved for the soil analysis before any treatment. The soil columns are then washed with distilled water to ensure that the pH of outgoing water from each column is neutral.

3.3 Soil Analysis

The soil used for chemical analysis was sieved in 2 mm sieve, and dried at 105°C. Several tests were conducted on soil before any treatment with pharmaceuticals.

3.3.1 Soil Texture (Hydrometer Test)

The particle size distribution of a soil expresses the proportions of the various size classes (clay < 0.002 mm, silt 0.002-0.02 mm and sand 0.02-2.0 mm particle size), commonly represented by weight percentages of the total soil. The proportions of these fractions are determined by Hydrometer method (Bouyoucos 1962) based on the Stokes`s Law which states that the rate of fall of particles in a suspension is directly proportional to their size [61, 62]. The soil was sieved using 2mm sieve, and dried at 105°C for 24 hours by Elle oven. The soil texture was determined by ASTM 152-H hydrometer.

3.3.2 Moisture

The results of soil analysis were calculated on the basis of an oven dried sample weight. Therefore, the moisture analysis was executed before

any other analysis. The results on the basis of the air-dry weight were multiplied by a moisture correction factor (mcf).

A porcelain crucible was placed in Ari J. Levy oven at a temperature of 105° C and it was left for 2 hours, then cooled down to room temperature in a desiccator, the weight of the empty crucible was recorded. Ten grams of soil sample were weighed in the crucible; the crucible was placed for 12 hours in the oven at 105° C. Then cooled down to room temperature in a desiccator and reweighed again. The moisture content (M) & moisture correction factor (mcf) were calculated using the following equations [63, 64]:

$$M\ (\text{moisture content})\ \% = \frac{(B-C)\times 100\%}{(C-A)}$$

Where:

A: Empty crucible weight

B: Sample + Crucible weight before drying

C: Sample + Crucible weight after drying

$$\text{mcf (moisture correction factor)} = \frac{100+M\ (\%)}{100}$$

3.3.3 pH

Twenty five grams of an oven dried - sieved soil were weighed, transferred into 100 ml beaker; 50 ml of distilled water were added while stirring for one hour using Freed Electric magnetic stirrer. The pH meter (Metrohm, 827 pH Lab–Omega symbol) was calibrated using pH buffer 4.0, 7.0 & 9.0, then the pH of suspension was measured [64,65].

3.3.4 Organic Carbon (Walkely and Black 1934)

One gram of the sieved soil was weighed & transferred into 500 ml conical flask, 10 ml of 1N $K_2Cr_2O_7$ and 20 ml of conc. H_2SO_4 were added, swirled carefully then it was let to stand for 30 minutes. Slowly 200 ml distilled water and 10 ml H_3PO_4 were added. Then 1 ml of diphenylamine indicator was added and the resulted suspension was titrated against 0.5 N ferrous ammonium sulphate solution until green color started appearing indicating the end point. The carbon content was calculated using the following equation [66, 67]:

$$\text{Organic Carbon (\%)} = \frac{10(B-S) \times 0.39 \times mcf}{B \times W}$$

Where,

B = ml of ferrous ammonium sulphate solution used for blank.

S = ml of ferrous ammonium sulphate solution used for sample.

mcf = moisture correction factor.

W = sample weight (g).

0.39 = conversion factor (including a correction factor for a supposed 70% oxidation of organic carbon.

% of organic matter = 1.72 X % of organic carbon

3.3.5 Total Nitrogen (Kjeldhal Method)

Nitrogen in soil/sediments is mostly present in the organic form with small quantities of ammonium and nitrate. This method measures only organic and ammoniacal form, therefore nitrate is excluded. The sample is digested in a catalyst mixture which converts all N into ammonium sulphate. The distillation of ammonia (librated after sodium hydroxide is added to ammonium sulphate), over boric acid and titrated against standardized acid to determine nitrogen.

One gram of the soil sample was placed in digestion tube. A 10 ml sample of conc. H_2SO_4 was added & swirled until the acid was mixed with the sample. The sample was allowed to cool. Two & a half grams of a catalyst mixture (containing K_2SO_4, $CuSO_4.5H_2O$, TiO_2 & anatase) was added & the mixture was heated until the digestion mixture becomes clear, and then boiled gently for 5 hrs. The mixture was allowed to cool & 20 ml of deionized water were added slowly with shaking. The tube was swirled to bring any insoluble material into the suspension then the tube was transferred to the distillation apparatus. The tube was rinsed three times

with water to complete the transfer. A 5 ml sample of boric acid (20 gm/Lt) was added into 250 ml conical flask, and the flask was placed under the condenser of the distillation apparatus in such a way that the end of the condenser was dipped into the solution. Twenty ml of NaOH (10 mol/Lt) was added to the funnel of the apparatus and the alkali was run slowly into the distillation chamber. About 100 ml of the condensate was distilled. The condenser was rinsed and few drops of indicator (0.1 g of bromocresol green, 0.02 g of methyl red in 100 ml ethanol) was added to the distillate & titrated with sulfuric acid to the violet end point. The percent nitrogen was calculated using the following equations [68]:

$$\% N = \frac{(V_1 - V_0) \times c(H^+) \times M_N}{m \times m_t} \times 100 \%$$

Where:

V_1: is volume, in ml, of the H_2SO_4 used in the titration of soil sample.

V_0: is volume, in ml, of the H_2SO_4 used in the titration of blank test.

$c(H^+)$: is the concentration of H^+ in the H_2SO_4 in mol/Lt (e.g 0.01 mol/Lt of H_2SO_4 is used, $c(H^+) = 0.02$ mol / Lt).

M_N: is the molar mass of N, in g/mol (= 14)

m: is the mass of the test sample

m_t: is the dry residue, expressed as g/100gm on the basis of oven dried material.

3.4 Calibration Curves

A standard calibration curves for both oxytetracycline & doxycycline were performed by preparing diluted solutions of oxytetracycline HCl & doxycycline HCl standards, both were purchased from KEMPEX, Holland.

100 mg of oxytetracycline HCl reference standard & 100 mg of doxycycline HCl reference standard were accurately weighed each of which alone, transferred into 100 ml volumetric flasks, distilled water was added to volume & stirred until completely dissolved. Several dilutions were made of each of them by taking 1ml, 2ml, 3ml, 4ml & 5ml from stock solution & transferred into 50 ml volumetric flasks. Distilled water was added to volume. Absorbance readings were recorded at 353 nm for oxytetracycline HCl & at 270 nm for doxycycline HCl.

3.5 Optimum Time for Oxytetracycline & Doxycycline Adsorption onto Soil

The purpose of this task is to determine the optimum time for the process of adsorption of both oxytetracycline HCL & doxycycline HCl onto soil to reach equilibrium. Two samples for oxytetracycline HCl solution were prepared & another two samples for doxycycline HCl, all four samples were prepared in 125 ml Erlenmeyer flask containing 5 grams of oven dried sieved soil, & 50 ml of 0.005% (v/v) of each tetracycline solution. All samples were covered with Teflon screw caps & mounted on Comfort Hetro Master Shaker at room temperature. All

samples were kept for 1, 2, 4, 6, 12, 24 & 36 hrs. Soil particles were allowed to settle then centrifuged using Hermel Z200A Centrifuge for 3000 rpm for 10 mins. After centrifuging, absorbance readings were recorded at 353 nm for oxytetracycline HCl & 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.

3.6 Isotherms

The most widely used equation to fit empirical data from solute – solvent adsorbent system is the Freundlich equation. Due to its simplicity & versatility in fitting data from systems, Freundlich relation ship 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% & 0.012% (w/v) of each oxytetracycline HCl & doxycycline HCl solutions were prepared, each in 125 ml Erlenmeyer flask, 5 grams of oven-dried sieved sample were added to each flask, 50 ml of each concentration for each substance were added to each flask, all samples were covered with Teflon screw caps & mounted on Comfort Hetro Master Shaker for 24 hrs. Soil was let to settle, & centrifuged at 3000 rpm for 10 mins. Absorbance readings were recorded at 353 nm for oxytetracycline HCl sample solutions & at 270 nm for doxycycline HCl sample solutions using UV-1601 PC, SHIMADZU spectrophotometer.

3.7 Polluting Soil with Oxytetracycline HCl & Doxycycline HCl

Seven columns were prepared for the pollution process; each was labeled according to the pollutant type & its quantity. The first column was considered as 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 gm of oxytetracycline HCl/ Lt) was prepared & added to the column, therefore it was labeled (OTC 1), the third column contained 7.5 gm of Oxin 50% powder / Lt (Oxin 50% contained 500mg/gm of oxytetracycline HCl, a product of the Palestinian Company for Veterinary Pharmaceuticals, Ramallah.), the column was labeled (OTC 2), the forth column, contained 15 gm of Oxin 50% powder / Lt 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 gm/Lt) of doxycycline HCl raw material and the column was labeled (DOX 1). The second one, was polluted with a solution containing (7.5 gm of Doxinal 10% powder /Lt) added to the column (Doxinal 10% contained 100 mg / gm of doxycycline as HCl, a product of Palestinian Company for Veterinary Pharmaceuticals) & labeled as (DOX 2). The last one contained a solution of 15 gm of Doxinal 10% powder / Lt, 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 & DOX 2 & thirty

times in OTC3 & DOX 3. The addition of pharmaceutical quantities was based on their dosages printed on labels of each drug. And It should be mentioned that doubling doses were a result of the frequent use of medications, and the dosages are approved by the Department of Drug Control in the Palestinian Ministry of Health.

3.8 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 Lt to each soil column. Soil columns were left for 24 hrs to ensure a complete adsorption process to soil.

3.9 Collecting & Storage of Soil & Leachate Water Samples

Water samples were collected & 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 [70].

Each soil column was divided into three zones (0-20 cm, 20-60 cm & 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).

3.10 Instrumentation

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

3.11 Polluted Soil Analysis

Soil samples were classified by region, from which the samples were taken; oxytetracycline HCl & doxycycline HCl absorbance readings were measured from 0-20 cm, 20-60 cm & from 60-100 cm in blank, OTC 1, OTC 2, OTC 3, DOX 1, DOX 2 & 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 & stirred for 30 minutes using Freed Electric magnetic stirrer, the suspension was filtered through Whatman filter papers no. 42, quartz cells were used during analysis.

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

potassium dichromate is titrated against ferrous ammonium sulphate. One g of polluted soil was weighed & transferred into a 500 ml conical flask, 10 ml of (1 N) $K_2Cr_2O_7$ & 20 ml of conc. H_2SO_4 were added. Swirled carefully and it was allowed to stand for 30 minutes. Slowly 200 ml of distilled water & 10 ml of H_3PO_4 were added. Then 1 ml of diphenylamine indicator was added and the solution was titrated against 0.5 N ferrous ammonium sulphate solution until green color started appearing indicating the end point. The organic carbon was calculated according to following equation [64, 65]:

$$\text{Organic Carbon (\%)} = \frac{10(B-S) \times 0.39 \times mcf}{B \times W}$$

Where

B = ml of ferrous ammonium sulphate solution used for blank.

S = ml of ferrous ammonium sulphate solution used for sample.

mcf = moisture correction factor.

W = sample weight (g).

0.39 = conversion factor (including a correction factor for a supposed 70% oxidation of organic carbon.

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 & 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 & DOX 2, twenty grams of polluted soil was weighed and transferred into 250 ml conical flask, 100 ml of distilled water & 1 gm of $MgCl_2.7H_2O$ (purchased from Merk, analytical grade) were added. 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.

3.12 Polluted Water Analysis

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

Chapter Four
RESULTS & 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 & fate of oxytetracycline HCl & doxycycline HCl as examples of tetracyclines in soil & underground water including their adsorption in soil.

4.1 Soil

Samples of soil were analyzed in order to evaluate the soil texture, moisture, organic matter, pH & nitrogen content. Table 4.1 presents the results obtained for these tests (each result was the average of three readings obtained & all calculations in this chapter were based on dried basis). And the Fig 4.1 shows a graph obtained for the hydrometer test for the soil sample.

Table (4.1): Soil texture, moisture content, moisture correction factor, pH, organic carbon, organic matter & 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
pH	7.13
Organic Carbon %	2.45%
Organic Matter %	4.21%
Nitrogen Content	0.155%

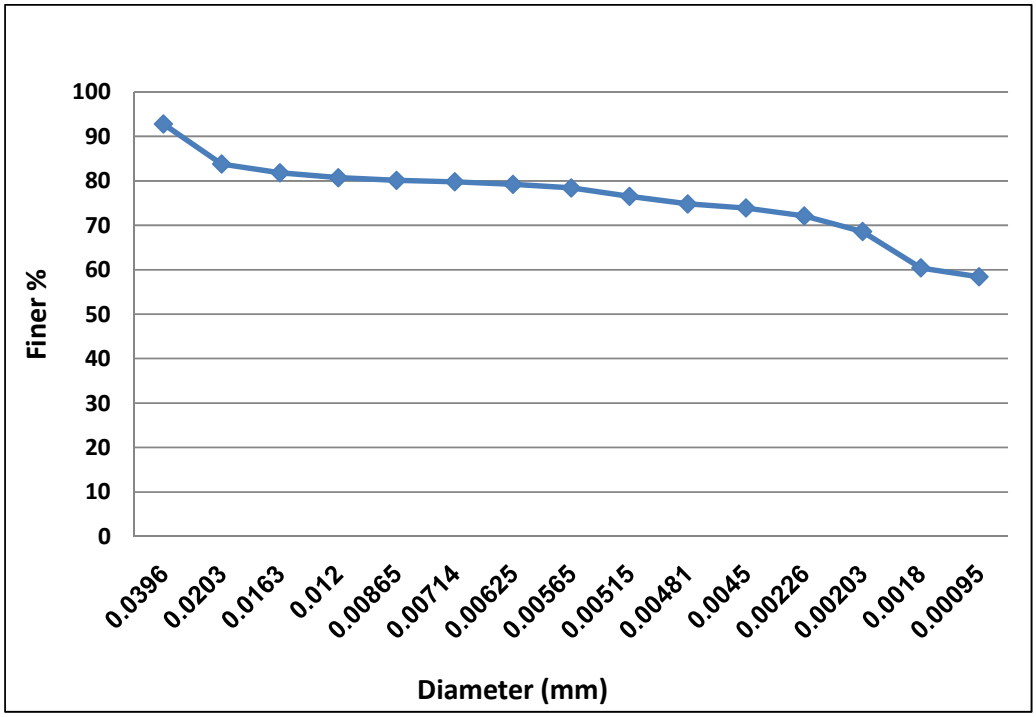


Fig. (4.1): Particle Size Distribution Curve (Hydrometer Test) 71.6% clay, 6.16% silt & 22.24% sand

4.2 pH Measurements for the Leachate Water Before & After Pollution

After the washing process with distilled water was done, pH readings for the leachate water were recorded before addition of pharmaceuticals in table 4.2.

Table (4.2): 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

From the above table, it's obviously 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 & doxycycline HCl have an acidic character after dissolving in distilled water, therefore after pharmaceuticals addition, soil media was expected to be slightly acidic since both pharmaceuticals used were as the hydrochloride derivative.

According to other researches [42,71] 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 & basic media, and it is greatly dependent on the pH of soil.

4.3 Optimum Time for Oxytetracycline HCl & Doxycycline HCl Adsorption onto Soil

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

Tables (4.4) & (4.5) shows concentrations (Conc.) of oxytetracycline & doxycycline solutions after 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 & 36 hours) equilibrium occurred after 24 hours of

adsorption for both oxytetracycline HCl & doxycycline HCl. Figures from 4.2 - 4.5 contains plotted graphs of ln pharmaceutical concentration vs time intervals.

Table (4.3): Concentrations of oxytetracycline HCl solution at different times

Time (hours)	Conc. of oxytetracycline HCl (mol/L) in the first sample	Conc. of oxytetracycline HCl (mol/L) in the second sample
1	1.58×10^{-5}	2.26×10^{-5}
2	1.42×10^{-5}	1.39×10^{-5}
4	1.17×10^{-5}	1.22×10^{-5}
6	9.75×10^{-6}	1.06×10^{-5}
12	9.12×10^{-6}	9.52×10^{-6}
24	8.16×10^{-6}	9.03×10^{-6}
36	8.15×10^{-6}	9.04×10^{-6}

Table (4.4): Concentrations of doxycycline HCl solution at different times

ime (hours)	Conc. of doxycycline HCl (mol/L) in first sample	Conc. of doxycycline HCl (mol/L) in the second sample
1	1.59×10^{-5}	1.65×10^{-5}
2	1.40×10^{-5}	0.98×10^{-5}
4	1.31×10^{-5}	0.87×10^{-5}
6	6.52×10^{-6}	7.96×10^{-6}
12	5.56×10^{-6}	7.62×10^{-6}
24	4.56×10^{-6}	6.77×10^{-6}
36	4.55×10^{-6}	6.76×10^{-6}

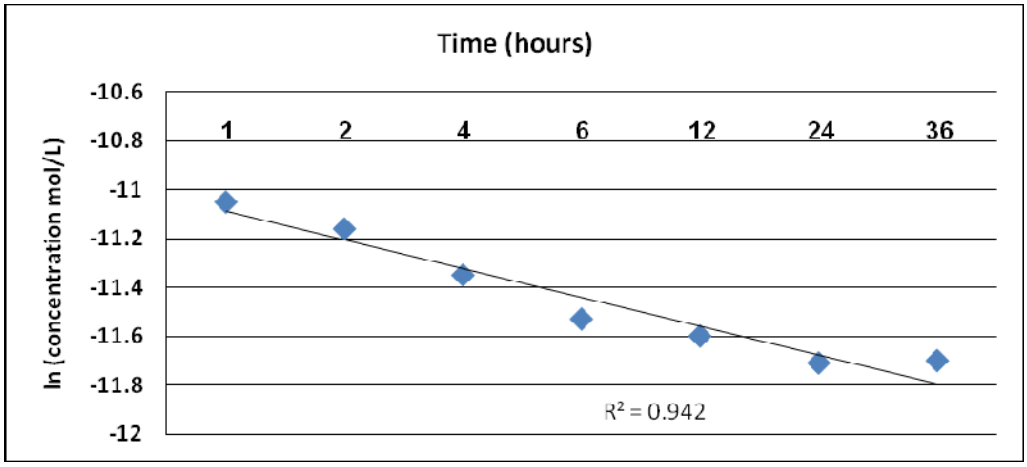


Fig. (4.2): Plot of ln concentration of oxytetracycline HCl vs time for

1st Sample

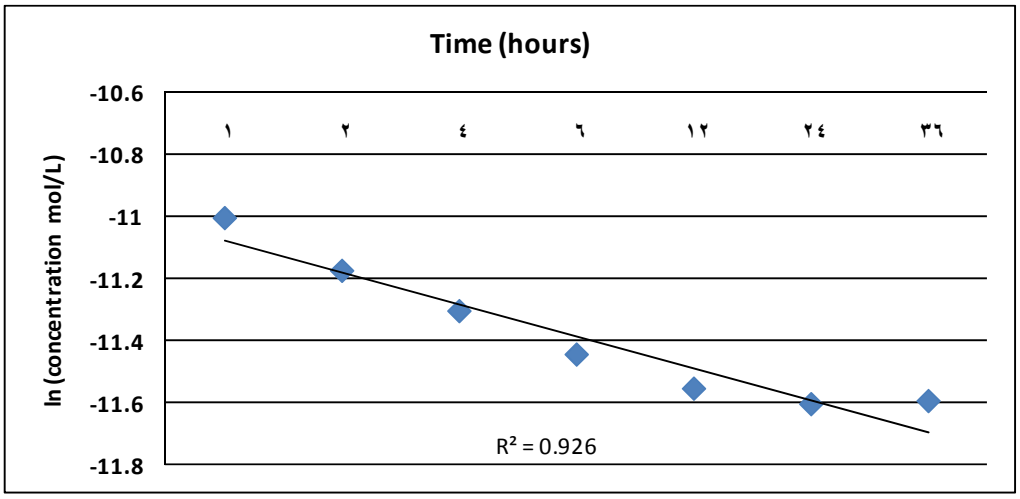


Fig. (4.3): Plot of ln concentration of oxytetracycline HCl vs time for

2nd Sample

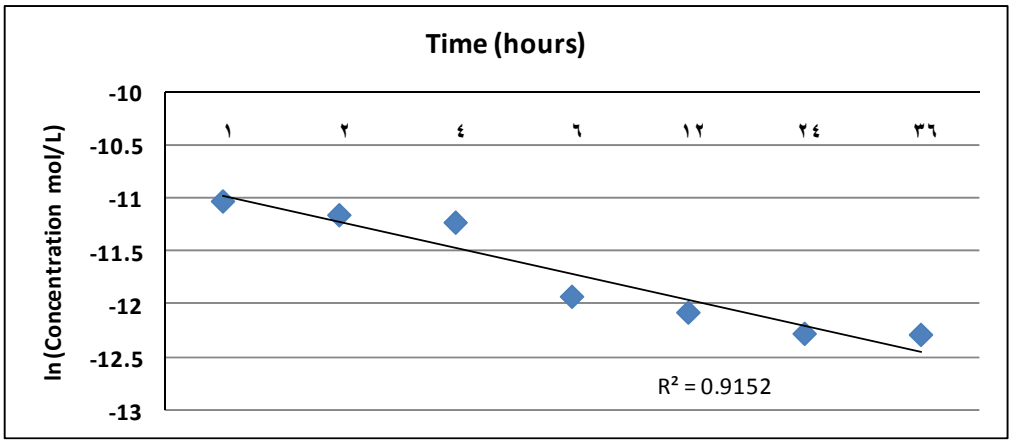


Fig. (4.4): Plot of ln concentration of doxycycline HCl vs time for

1st Sample

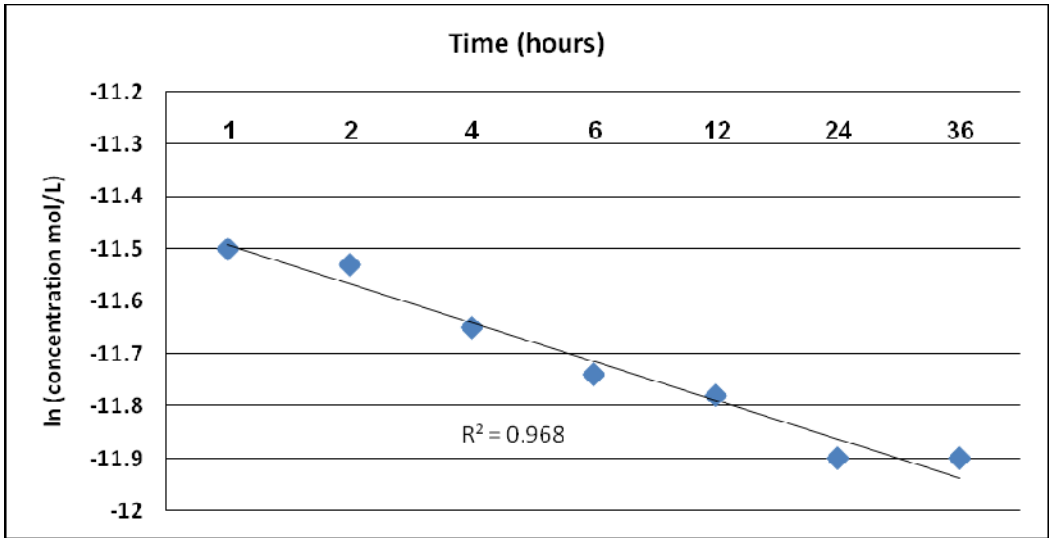


Fig. (4.5): Plot of ln concentration of doxycycline HCl vs time for

2^{nd} Sample

According to the above results, the optimum time for adsorption was after 24 hours of adsorption, both oxytetracycline & doxycycline adsorptions followed first order kinetic, R values (correlation coefficient) of both samples 1 & 2 for oxytetracycline & doxycycline readings were close to 1.

4.4 Adsorption Isotherms

The equilibrium adsorption data could be described by the Freundlich adsorption equation (4.1):

$$x/m = k\,(C_e)^{1/n} \qquad (4.1)$$

The Freundlich equation constants "k" & "n" could be obtained form the empirical Freundlich adsorption equation (4.1):

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.

The isotherm equilibrium results are shown in tables (4.6) (4.7), figures (4.6) & (4.7), as the amount adsorbed against the equilibrium concentrations after 24 hours of adsorption. Readings were recorded until 36 hours, no changes in concentrations were observed after 24 hours for all samples. All concentrations were converted into mol/L.

Table (4.5): Equilibrium concentrations (C_e) & amount of oxytetracycline HCl adsorbed per gm of soil (x/m)

x/m mol/gm of soil)	C_e (mol/L)
$9.54X10^{-4}$	$1.34\ X10^{-2}$
$1.31X10^{-3}$	$1.50\ X10^{-2}$
$2.13\ X10^{-3}$	$1.55\ X10^{-2}$
$2.95\ X10^{-3}$	$1.60\ X10^{-2}$
$3.71\ X10^{-3}$	$1.71\ X10^{-2}$
$4.46\ X10^{-3}$	$1.92\ X10^{-2}$

Table (4.6): Equilibrium concentrations (C_e) & amount of doxycycline HCl adsorbed per gm of soil (x/m)

x/m (mol/gm of soil)	C_e (mol/L)
$7.24 X10^{-4}$	$5.63 X10^{-3}$
$1.51 X10^{-3}$	$7.35 X10^{-3}$
$2.34 X10^{-3}$	$8.89 X10^{-3}$
$3.16 X10^{-3}$	$1.02 X10^{-2}$
$3.89 X10^{-3}$	$1.23 X10^{-2}$
$4.78 X10^{-3}$	$1.42 X10^{-2}$

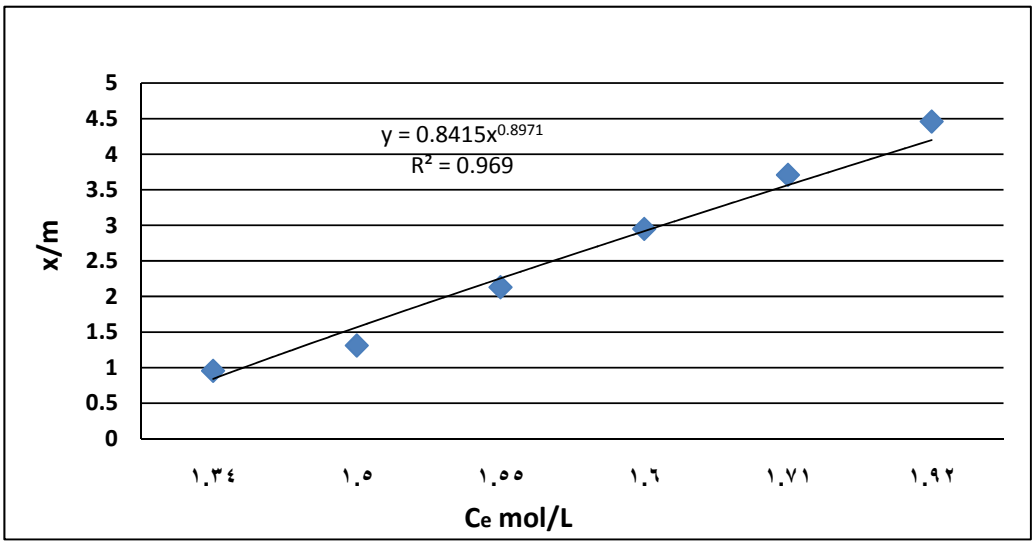


Fig. (4.6): Plot of C_e vs x/m for oxytetracycline HCl

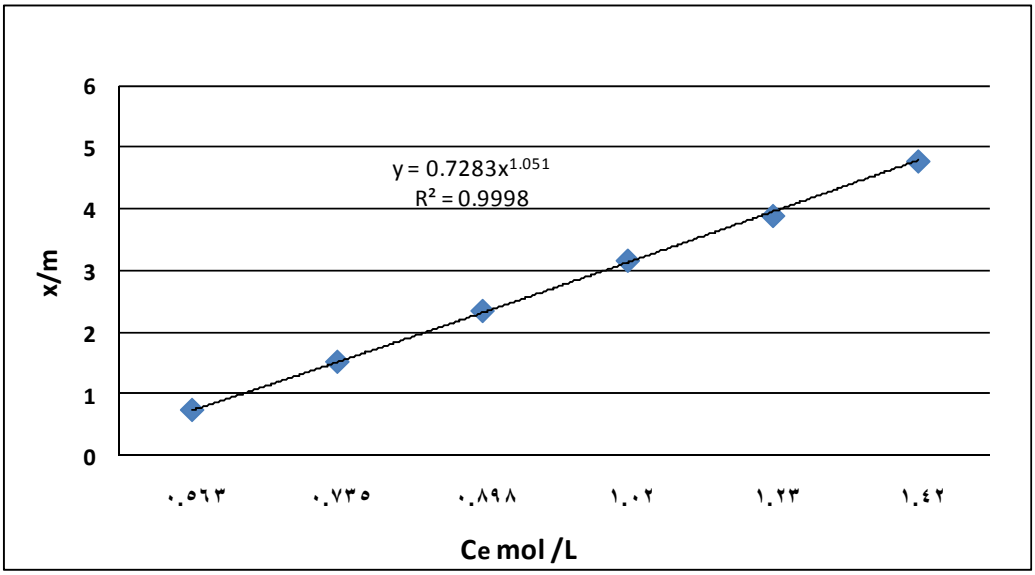


Fig. (4.7): Plot of C_e vs x/m for doxycycline HCl

Freundlich isotherm constants (k & n) for oxytetracycline HCl & doxycycline HCl & the correlation coefficient "R" were obtained from Figures 4.6 & 4.7 and listed in table 4.8.

Table (4.7): 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 4.8, n values for both oxytetracycline HCl & doxycycline HCl were found to be close to 1.

4.5 The Effect of Organic Matter

Organic matter influences physical & chemical properties of soil often to critical extent. Organic matter is essential to coarse-grained materials for providing nitrogen and higher cation exchange capacities [72].

Tables 4.9 & 4.10, shows that the organic matter content in the soil used in this study ranges 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 & doxycycline HCl in blank, OTC1, OTC2, OTC 3, DOX1, DOX2 & DOX3, which was an evident for all results.

Comparison of soil depth & the content of organic matter with concentrations of both tetracyclines measured were plotted in figures 4.10 - 4.16.

All concentrations were plotted in the form of mol/Lt; they were calculated from the standard curves of oxytetracycline HCl & doxycycline HCl standard solutions, figures 4.8 & 4.9.

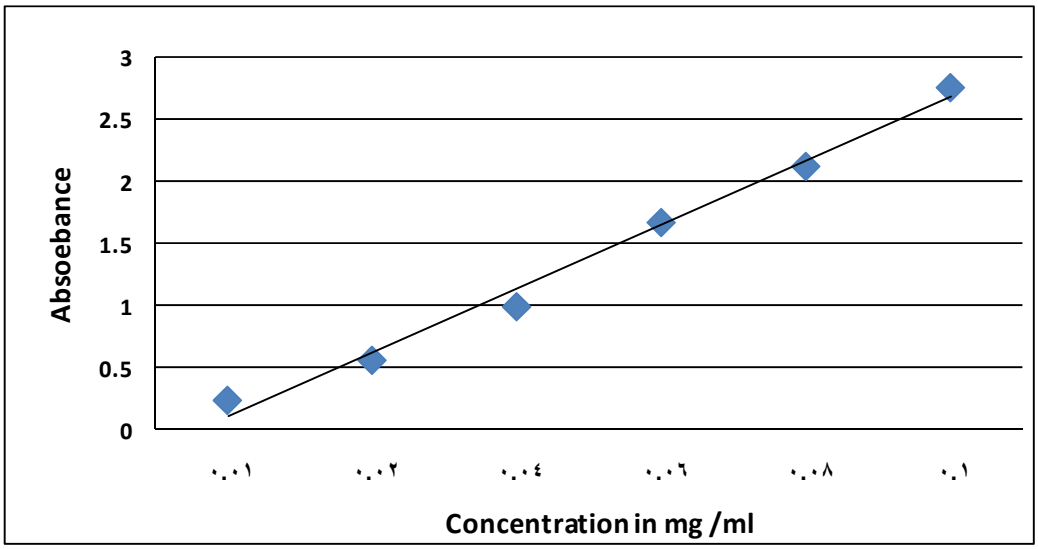


Fig. (4.8): Standard calibration curve for oxytetracycline HCl

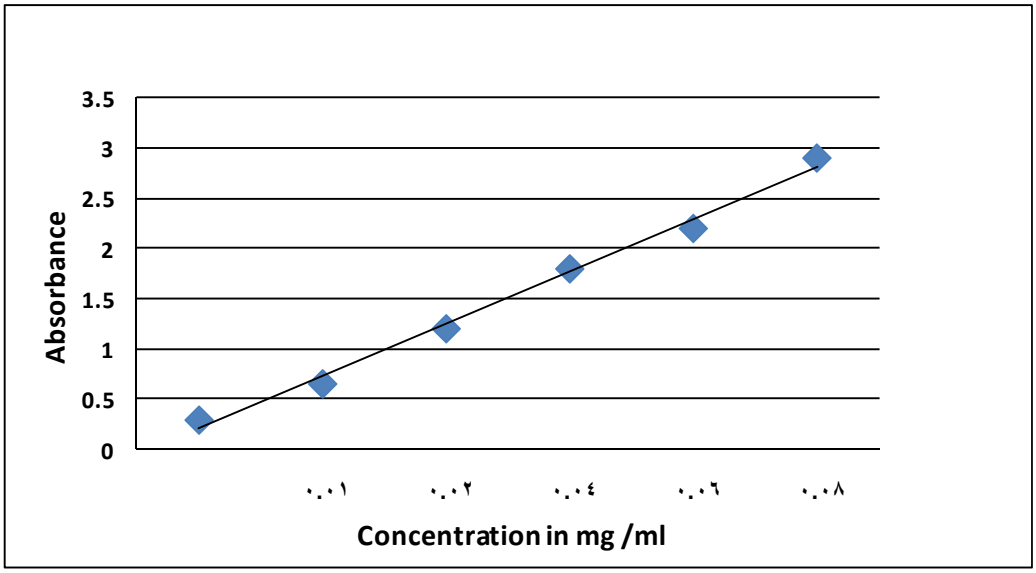


Fig. (4.9): Standard calibration curve for doxycycline HCl

Table (4.8): 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 (4.9): 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 %

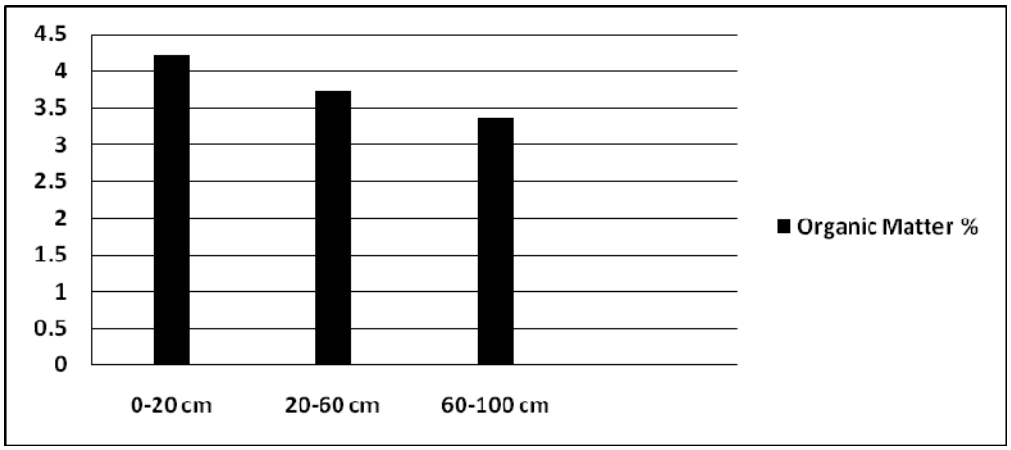


Fig. (4.10): Organic matter content in blank soil column, no traces for any of tetracyclines detected

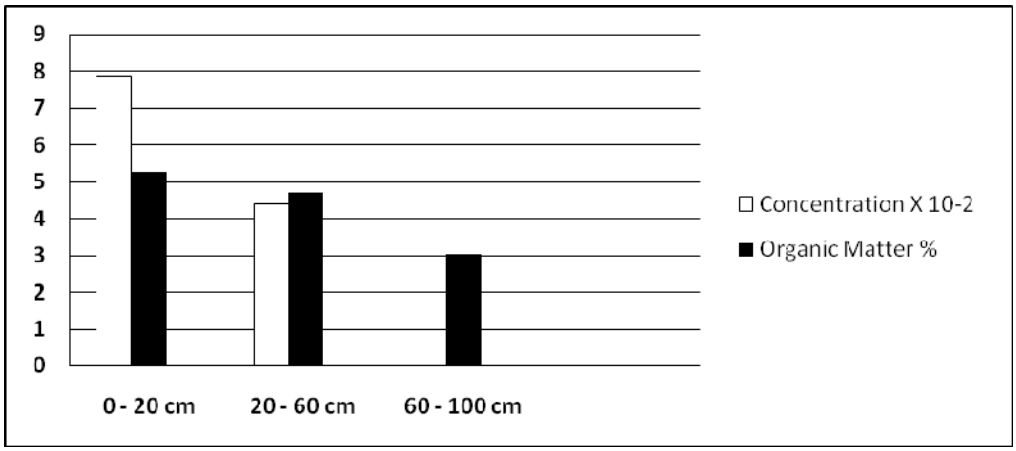


Fig. (4.11): Organic matter content in OTC 1 column & concentrations measured for oxytetracycline HCl

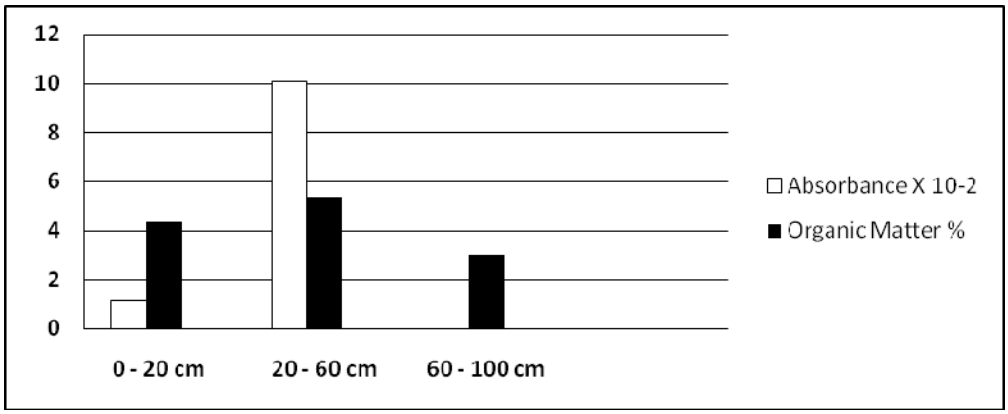


Fig. (4.12): Organic matter content in OTC 2 column & concentrations measured for oxytetracycline HCl

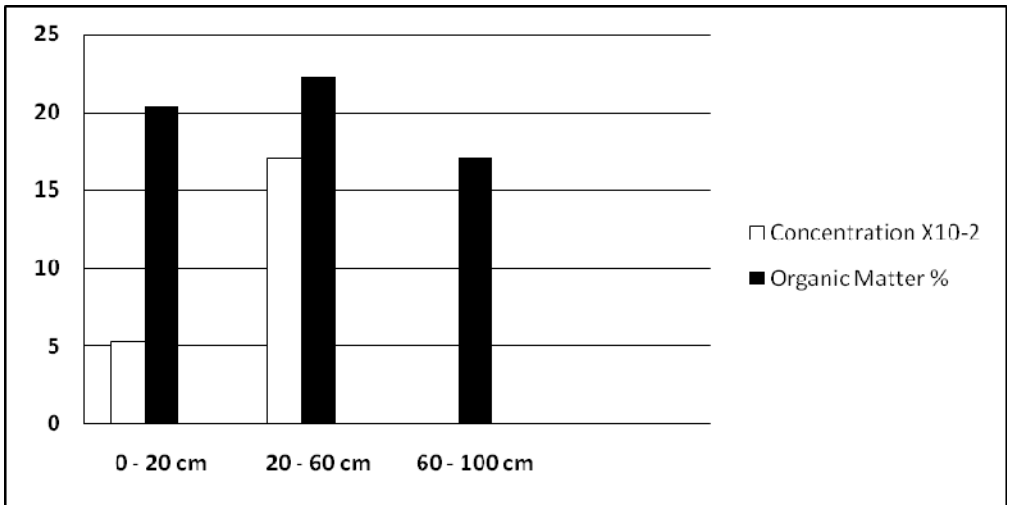


Fig. (4.13): Organic matter content in OTC 3 soil column & concentrations measured for oxytetracycline HCl

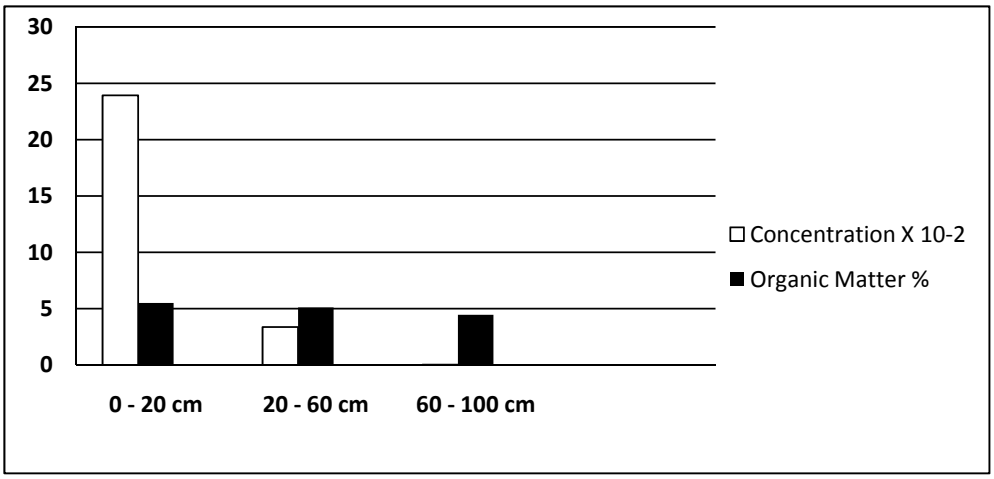


Fig. (4.14): Organic matter content in DOX 1 soil column & concentrations measured for doxycycline HCl

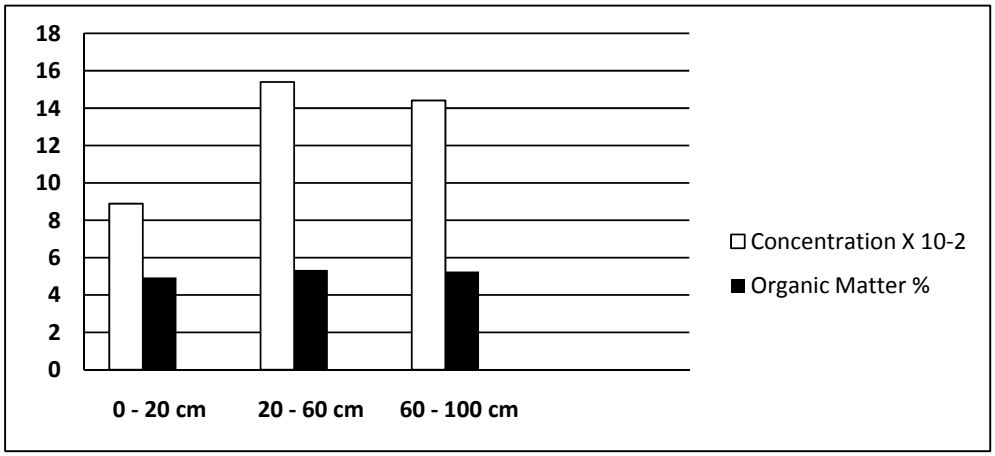


Fig. (4.15): Organic matter content in DOX 2 column & concentrations measured for doxycycline HCl

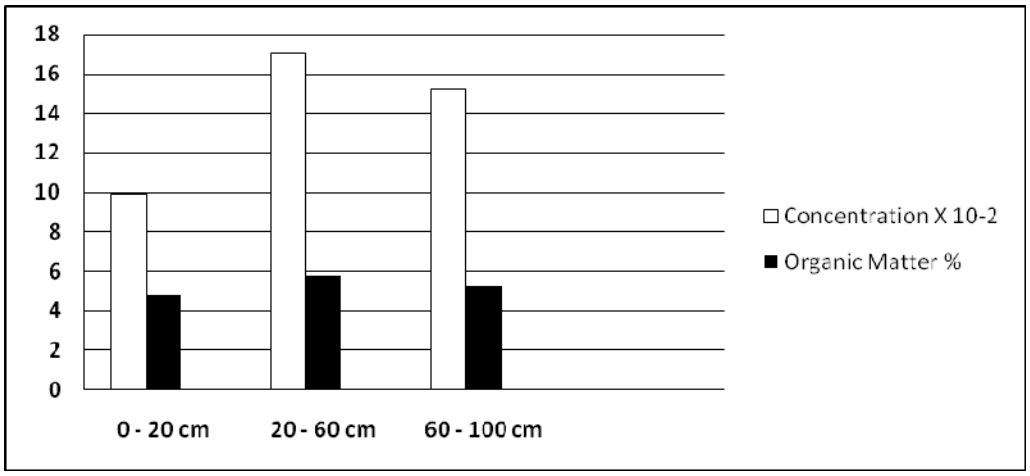


Fig. (4.16): Organic matter content in DOX 3 column & concentrations measured for doxycycline HCl

From the results of this part of research the role of organic matter content was noticeable; both pharmaceuticals were distributed along the columns. In OTC1 & DOX1 soil columns highest concentrations were obtained in area 0-20 cm were high percentage of organic matter was found. In addition chelating to surface metals can be another factor for the presence of large amounts of tetracyclines on surface, this was proven in researches [73,74].

In OTC2 & OTC3 soil columns higher concentrations were found in area 0-20 & 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 [75].

In DOX2 & DOX3 soil columns, doxycycline concentrations were distributed all over the columns, especially in areas from 40-60 cm & 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 has 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 [76]. Several investigations had shown that there is a major adsorption of tetracyclines by reference soil components, such as clays (Kulshestra et al. 2004) and hydrous oxides of soil (Figueroa and Mackay 2005; Gu and Karthikeyan 2005) [71].

4.6 Effect of $MgCl_2.7H_2O$ addition to soil

As known light, temperature, moisture & duration of storage influence the stability of tetracyclines [77]. 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 & 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 & were recorded in tables 4.9 & 4.10, and plotted against time in figures 4.17 – 4.18.

In many researches the effect of ionic strength on tetracycline adsorptions was studied, in order to determine the bioavailability of tetracyclines [78, 79], or stability of complexes formed. Adsorption of

tetracyclines and metals on soil minerals strongly affects their mobility. According to 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[80]. Another one suggested that calcium salts promoted oxytetracycline sorption at alkaline pHs likely by a surface-bridging mechanism [81]. Magnesium was chosen in this research as 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 deicer, in this way large quantities of $MgCl_2$ is used to de-ice roads. In addition magnesium chloride is also used in pharmaceutical industry for its chelating ability & its low health risk, as other contaminants it can reach the soil & affect its natural characteristics. For the above mentioned reasons, magnesium chloride was chosen as an example of chelating metal that forms complexes with tetracyclines.

Table (4.10): Concentrations of oxytetracycline-Mg complex measured at 353 nm at room temperature

Time (hours)	Concentration (mol/L)
0	2.70×10^{-1}
24	2.70×10^{-1}
48	2.70×10^{-1}
72	2.70×10^{-1}
216	2.70×10^{-1}
348	1.31×10^{-1}

Table (4.11): Concentrations of doxycycline-Mg complex measured at 270 nm at room temperature

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

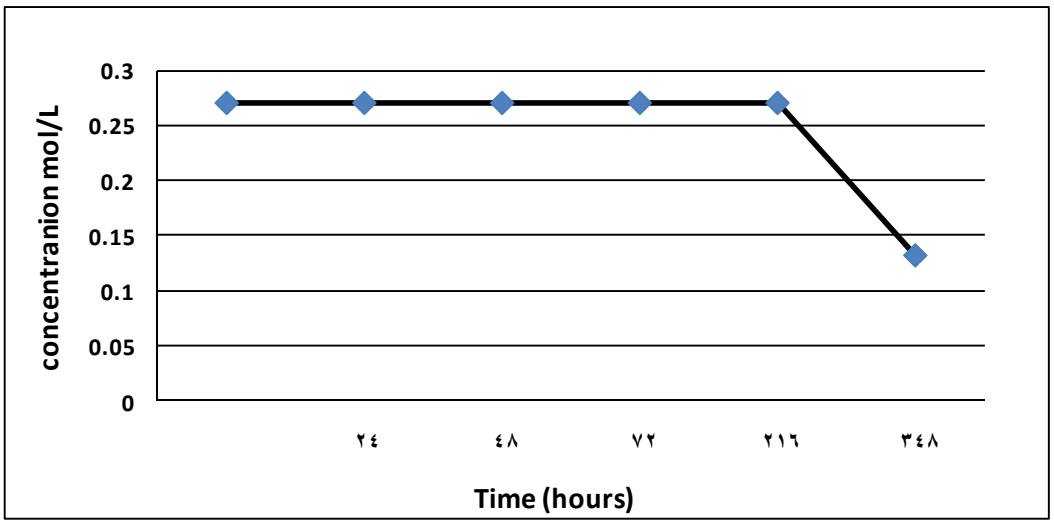


Fig. (4.17): Plot of concentration of oxytetracycline-Mg complex measured at 353 nm at room temperature

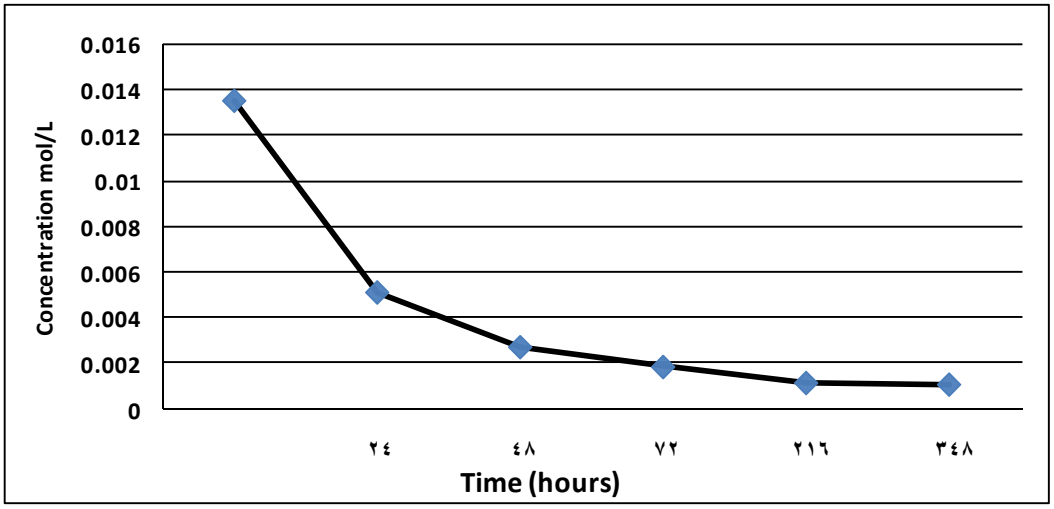


Fig. (4.18): Plot of concentration of doxycycline-Mg complex measured at 270 nm at room temperature

From the results of the previous experiment in section 4.6, the rate of hydrolysis & 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 are 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 [82].

4.7 Polluted Water Analysis

The leachate water that flowed from each soil column was kept in well closed HDPE containers, & stored in refrigerator (at 7°C), all were analyzed by UV-Vis spectrophotometer, absorbance readings were recorded, then transformed into concentrations (mol/L) using standard calibration curves, then all were plotted against time. Figures from 4.19 - 4.24 shows a plot of ln [A], where A is the concentration of tetracycline (mol/L) for every absorbance reading from each column measured at different times, straight lines were obtained for both oxytetracycline HCl & doxycycline HCl, which was the indication of first order hydrolysis reaction.

Tables 4.13 - 4.18 shows the concentration [A] measured in (mol/L) versus time in hours.

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

Table (4.12): Measured concentrations of polluted water flowed from OTC1 versus time

Time (hours)	[A] mol/L	ln[A]
0	7.93×10^{-3}	-4.84
3	7.84×10^{-3}	-4.84
6	7.45×10^{-3}	-4.89
24	6.64×10^{-3}	-5.01
48	6.23×10^{-3}	-5.07
72	5.83×10^{-3}	-5.14
96	5.43×10^{-3}	-5.24
120	4.42×10^{-3}	-5.42
144	4.22×10^{-3}	-5.46
168	4.02×10^{-3}	-5.51
336	2.01×10^{-3}	-6.02
504	1.06×10^{-3}	-6.84
672	0.8×10^{-3}	-7.13

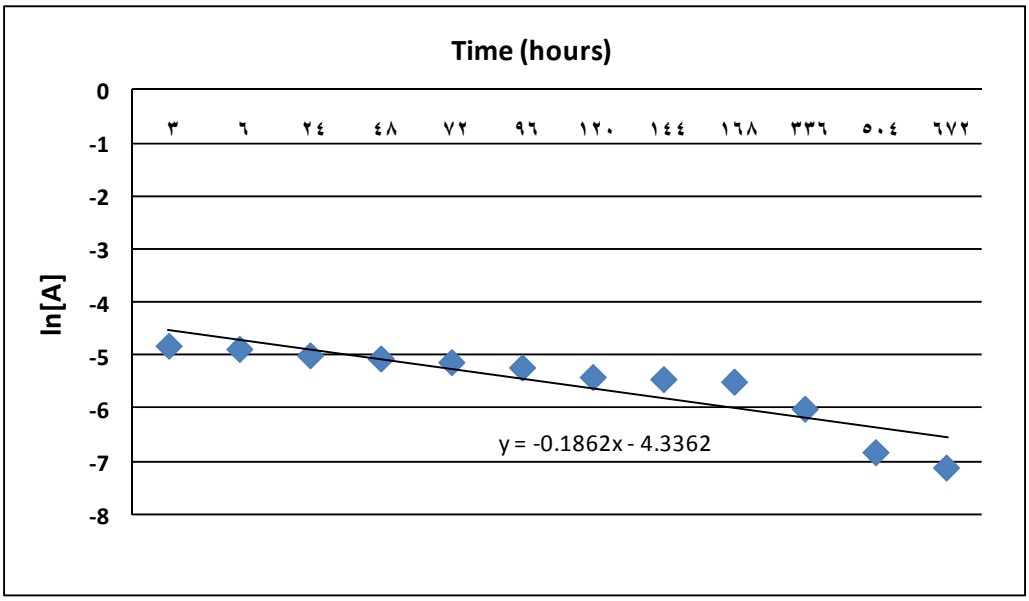


Fig. (4.19): ln[A] versus time for polluted water flowed from OTC1

Table (4.13): Measured concentrations of polluted water flowed from OTC2 versus time

Time (hours)	[A] mol/L	ln[A]
0	4.40×10^{-3}	-5.426
3	4.20×10^{-3}	-5.472
6	4.02×10^{-3}	-5.516
24	2.81×10^{-3}	-5.874
48	2.01×10^{-3}	-6.209
72	1.97×10^{-3}	-6.229
96	1.95×10^{-3}	-6.239
120	1.89×10^{-3}	-6.271
144	1.87×10^{-3}	-6.281
168	1.84×10^{-3}	-6.297
336	1.79×10^{-3}	-6.325
504	1.79×10^{-3}	-6.325
672	1.72×10^{-3}	-6.365

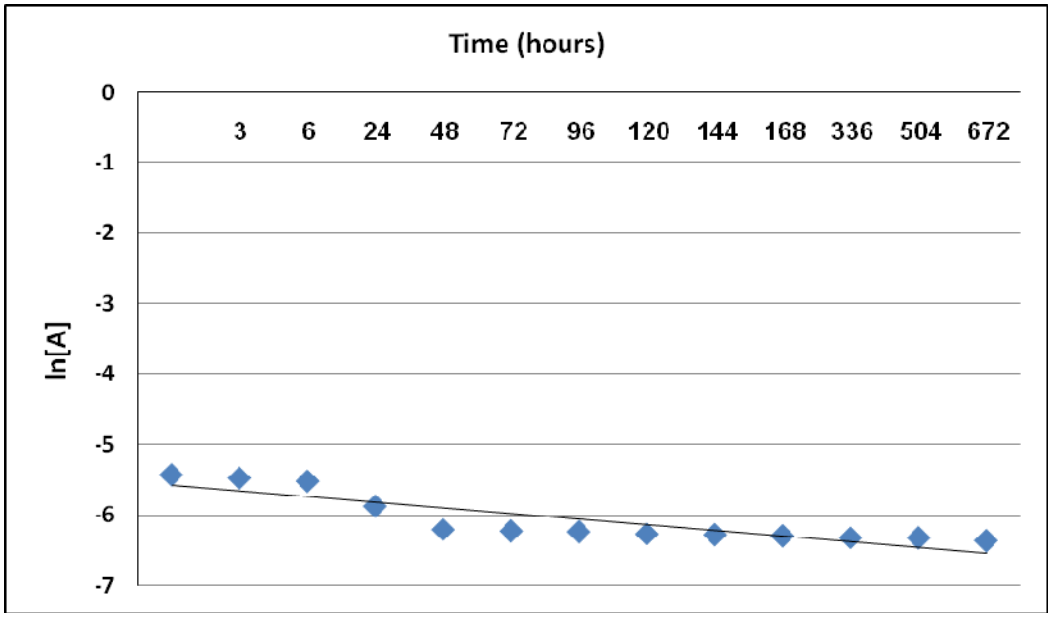


Fig. (4.20): ln[A] versus time for polluted water flowed from OTC2

Table (4.14): Measured concentrations of polluted water flowed from OTC3 versus time

Time (hours)	[A] mol/L	ln[A]
0	1.48×10^{-3}	-6.50
3	1.44×10^{-3}	-6.54
6	1.20×10^{-3}	-6.72
24	1.13×10^{-3}	-6.78
48	1.01×10^{-3}	-6.89
72	0.79×10^{-3}	-7.14
96	0.62×10^{-3}	-7.38
120	0.52×10^{-3}	-7.56
144	0.33×10^{-3}	-8.01
168	0.21×10^{-3}	-8.46
336	0.15×10^{-3}	-8.80
504	0.09×10^{-3}	-9.31
672	0.04×10^{-3}	-10.12

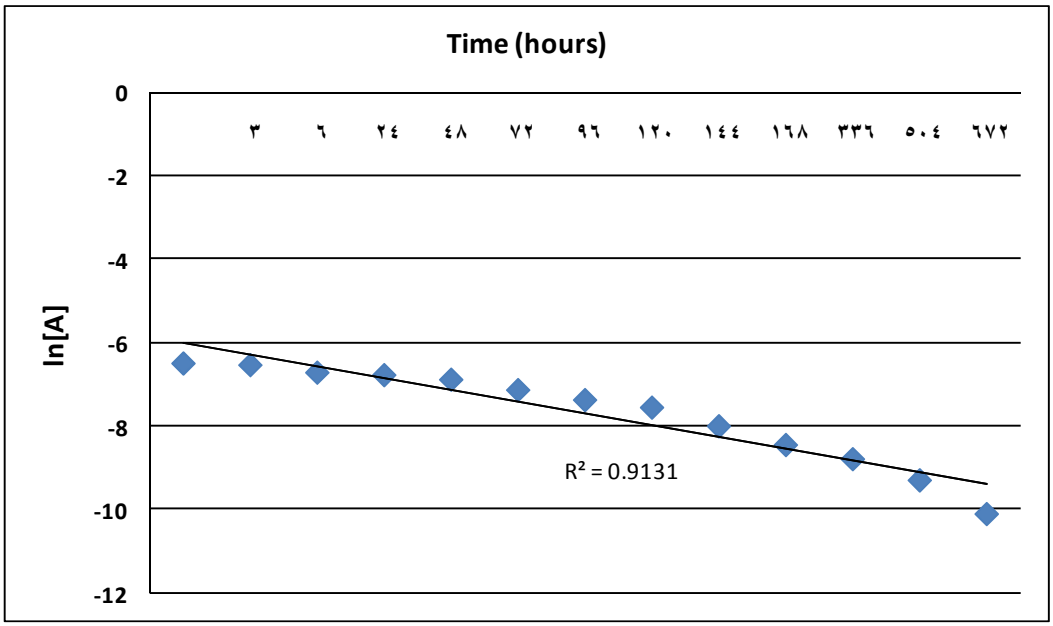


Fig. (4.21): ln[A] versus time for polluted water flowed from OTC3

Table (4.15): Measured concentrations of polluted water flowed from DOX1 versus time

Time (hours)	[A] mol/L	ln[A]
0	4.05×10^{-2}	-3.20
3	3.97×10^{-2}	-3.22
6	3.65×10^{-2}	-3.31
24	3.53×10^{-2}	-3.34
48	3.50×10^{-2}	-3.35
72	3.46×10^{-2}	-3.36
96	3.26×10^{-2}	-3.42
120	3.01×10^{-2}	-3.50
144	2.88×10^{-2}	-3.54
168	2.61×10^{-2}	-3.64
336	2.52×10^{-2}	-3.68
504	2.4×10^{-2}	-3.72
672	2.38×10^{-2}	-3.74

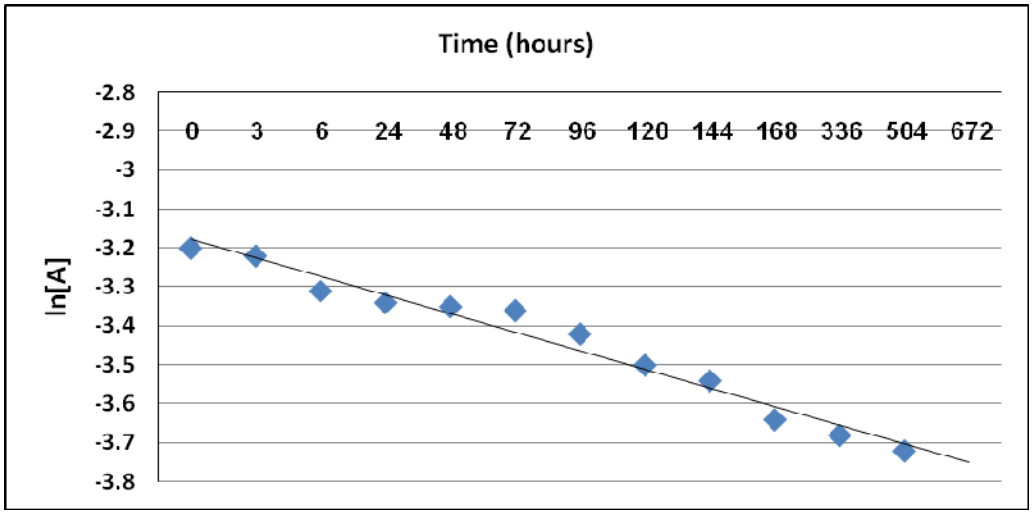


Fig. (4.22): ln[A] versus time for polluted water flowed from DOX1

Table (4.16): Measured concentrations of polluted water flowed from DOX2 versus time

Time (hours)	[A] mol/L	ln[A]
0	0.1438	-1.938
3	0.1432	-1.943
6	0.1331	-2.016
24	0.1212	-2.110
48	0.1114	-2.194
72	0.0962	-2.341
96	0.0919	-2.387
120	0.0914	-2.392
144	0.0881	-2.429
168	0.0875	-2.436
336	0.0825	-2.494
504	0.0801	-2.524
672	0.0793	-2.534

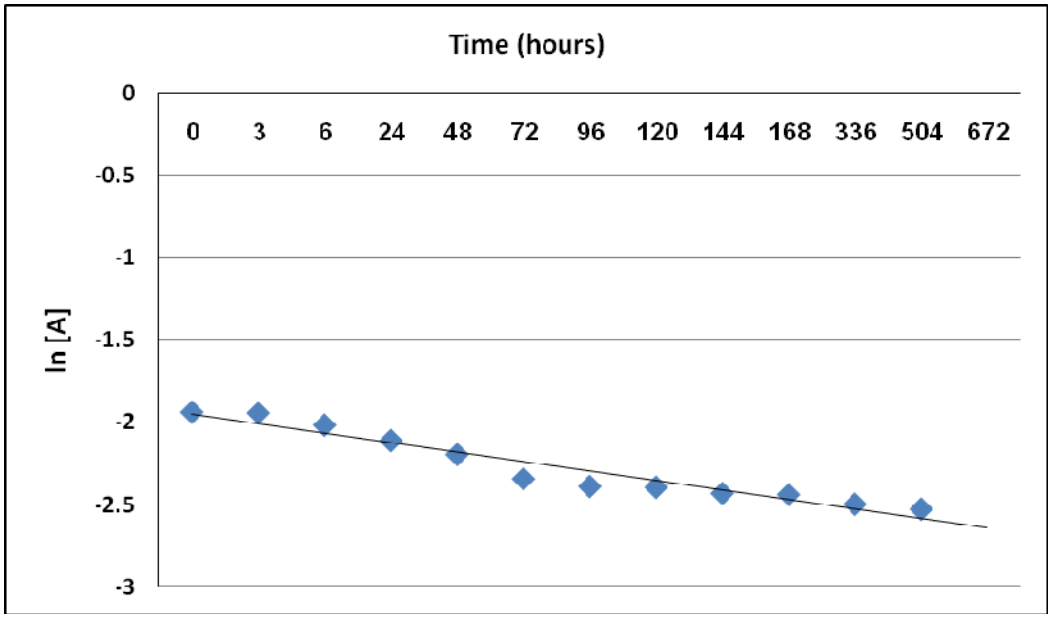


Fig. (4.23): ln[A] versus time for polluted water flowed from DOX2

Table (4.17): Measured concentrations of polluted water flowed from DOX3 versus time

Time (hours)	[A] mol/L	ln[A]
0	0.239	-1.431
3	0.238	-1.432
6	0.230	-1.469
24	0.221	-1.509
48	0.209	-1.565
72	0.196	-1.629
96	0.172	-1.760
120	0.165	-1.801
144	0.143	-1.944
168	0.136	-1.995
336	0.133	-2.017
504	0.129	-2.047
672	0.124	-2.087

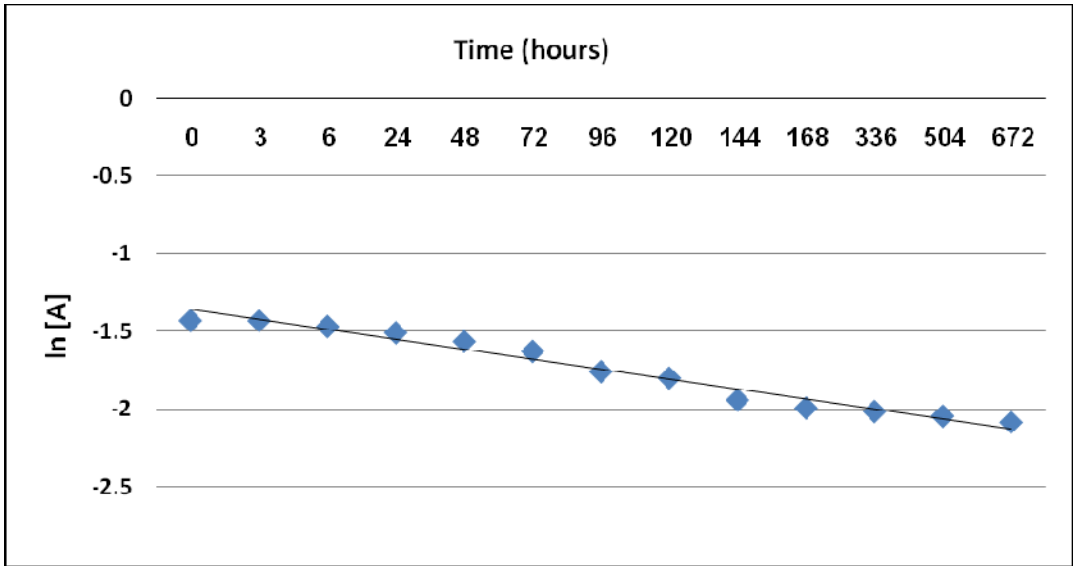


Fig. (4.24): ln[A] versus time for polluted water flowed from DOX3

As prescribed in the above tables, the concentration measured of oxytetracycline in polluted water was in OTC1 was the highest among the three OTC columns, which was another 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, in spite of the presence of organic matter, but the effect of hydrophilicity of doxycycline & its high mobility were dominant over the organic matter effect, which was an indication that doxycycline can reach under ground water more easily than oxytetracycline. Although oxytetracycline has low mobility in soil but it can be found in surface & under ground water [83]. And the presence of oxytetracycline in higher concentrations in soil layers is probable to be due to organic matter effect & chelation with metal ions.

Hydrolysis of oxytetracycline HCl & doxycycline HCl in polluted water followed first order kinetic as prescribed in the plotted graphs straight lines were obtained after plotting ln[A] vs time.

In other researches, kinetics of tetracyclines degradation follow first order rates [84] and known degradation products were used to confirm that degradation had occurred in polluted under ground water [85].

Conclusions & Recommendations

Conclusions

Pharmaceuticals have been found in soil & underground water in many countries, but little is known about the occurrence and the fate of them in the environment. Investigation of adsorption characteristics of antibiotics in soils is of great importance environmentally, because such a process is associated with the ecotoxicity, degradation, transportation, and bioaccumulation of antibiotics in the soil environment.

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 and investigations on the environmental effects of these antibiotics are necessary.

The adsorption of tetracycline antibiotics can occur via physical mechanisms such as hydrogen bonding, Vander Waals forces, and/or chemical mechanisms including cationic exchanges, protonation, electrostatic interactions, coordination, and complexation. Furthermore, the adsorption of tetracycline can be characterized by 2 processes of different kinetics: a fast initial adsorption to outer surfaces, followed by a slow penetration by slow diffusion into interlayers between clay minerals and micropores.

The adsorption isotherm curves have the C-type isotherm according to Giles classification & 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 physical properties such as solubility of the adsorbate has been found to affect Freundlich constant & thus adsorption tendency, as the degree of solubility increases Freundlich constant decreases.

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

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

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

Oxytetracycline HCl was more affected by the presence of organic matter than doxycycline HCl, this is due to high solubility & high mobility of doxycycline with water. Hydrophobicity & hydrophilicity of organic compounds can be considered another factor of controlling mobility inside soil layers & contributing in pharmaceutical pollution of under ground water.

Effect of complex formation may help in sustaining pharmaceutical residues inside soil matrix; this can affect the soil texture, pH & bacterial activity. Since 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.

Tetracyclines can reach to under ground water or surface water through several mechanisms, which may lead to bacterial resistance genes & pharmaceutical residues; Tetracyclines can form toxic residues after degradation & hydrolysis in water, as shown in this experiment the concentrations of both pharmaceuticals decreased with time due to degradation. Degradation followed first order rate for both tetracyclines, afterwards these degrades can be transferred to humans & wildlife.

Recommendations

To restrict pharmaceutical pollution, it is recommended to:

1-Use microbs in manure can minimize pharmaceutical pollution.

Waste from treated animals should be stored in a warm moist place for long as possible before spreading it into fields. This gives the beneficial soil microbes an opportunity to act on an antibiotic, before it has the chance to leach into soils & waterways.

2-Establishing Take Back Programs

Pharmacies & health communities accept unwanted & expired pharmaceuticals. These programs are now applied in Europe.

3-Green Pharmacy or getting back to Mother Nature

Using herbal medications can minimize pharmaceutical pollution, if they end up to soil or water systems, causing exposure to organisms that have been adapted to these products naturally.

4-More researches in this regard should be carried out:

- Further investigation into the fate of tetracyclines in the environment (e.g. degradation rates, local and global distribution, bioavailability).
- Further improvement and validation of the employed methods for the analysis of tetracyclines in soil, water and liquid manure.
- Development of methods or techniques to accelerate the degradation of tetracyclines in slurry.
- Development of analytical methods for other frequently used veterinary drugs including their metabolites (e.g. sulfonamides).
- Development of suitable ecotoxicological test methods, especially for antibiotics (acute effects / antibiotic resistance).
- Relevant case studies with realistic concentration range to perform environmental risk assessment.

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جامعة النجاح الوطنية
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مآل مادتي أوكسي تتراسايكلين ودوكسي سايكلين في التربة والمياه الجوفية

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لما سميح محمد عورتاني

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قدمت هذه الأطروحة استكمالا لمتطلبات الحصول على درجة الماجستير في الكيمياء من كلية الدراسات العليا في جامعة النجاح الوطنية، نابلس – فلسطين.
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الملخص

يعتبر التلوث الدوائي من أخطر أنواع التلوث البيئي الذي بدأ يظهر جليا ويتصدر الأبحاث والدراسات البيئية في الوقت الحاضر، وذلك لعظم تأثيره على الحياة المائية والتربة والمياه الجوفية، ليمتد تأثيره ويصل إلى النباتات والحيوانات والإنسان– الذي يكون هو السبب المباشر وغير المباشر في حدوث هذا النوع من التلوث. في هذه الدراسة تم اختيار نوعين من المضادات البكتيرية وهما مادتا أوكسيتتراسايكلين ودوكسي سايكلين ، اللتان يتم تداولهما هنا في السوق الفلسطينية سواء في نطاق صناعة الأدوية البشرية أو البيطرية، وفي هذا البحث تم دراسة السلوك الإدمصاصي لهما في التربة، وتأثير وجود المادة العضوية على عملية الإدمصاص، وكذلك تأثير وجود مادة كلورات المغنيسيوم سباعية التميه على إدمصاصهما في التربة الملوثة ، وكذلك دراسة تأثيرهما على المياه الجوفية وخصائصها، وقد استخدم جهاز الامتصاص الطيفي للأشعة فوق البنفسجية والضوء المرئي (UV-Vis Spectrophotometer) في هذه الدراسة. وقد بينت النتائج أن زيادة المادة العضوية يزيد من عملية الادمصاص لمادة أوكسي تتراسايكلين أكثر من مادة دوكسي سايكلين، كما بينت أن تكوين معقد اوكسي تتراسايكلين مع أيون المغنيسيوم كان أكثر ثباتا من معقد دوكسي سايكلين مع المغنيسيوم. كما بينت الدراسة وجود تركيز أعلى لمادة دوكسي سايكلين في المياه المترشحة من التربة من تلك المترشحة من مادة أوكسي تتراسايكلين وذلك بسبب ذائبية دوكسي سايكلين العالية في الماء. كما أظهرت أيضا تناقصا في تركيز المادتين مع

مرور الوقت في الماء المترشح بسبب تحللهما. إن تحلل هاتان المادتان في التربة وفي الماء من شأنه أن ينتج عنه مواد أخرى قد تكون ضارة، كما أن خطر بقائهما في التربة والمياه الجوفية من شأنه أن يزيد من مقاومة البكتيريا الموجودة في التربة لهما وبعبارة أخرى من شأنه أن يؤثر على خصائص التربة الطبيعية والمياه الجوفية كذلك.