

# Removal of the Oxytetracycline Antimicrobial from Aqueous Solution using Waste Tire Crumb Rubber

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## Abstract:

As of today we face serious problems of pollution, of which one is the excess of antimicrobial compounds (e.g. Oxytetracycline, OTC) and another is the stockpiles of used tires. Our research project evaluated the sorption behavior of OTC onto waste tire crumb rubber (WTCR) in aqueous solution; since previous studies showed that WTCR has a great potential for sorption of antimicrobial compounds. OTC samples from 25 to 150 mg/L (ppm) at neutral pH were placed in contact with 10 g/L of crumb rubber mesh 14-20 (2.45 mm of diameter) for 84 hours. The samples were collected at different intervals and analyzed by High Performance Liquid Chromatography with a Diode Array Detector (HPLC-DAD) at 353 nm using an Eclipse XDB-C8 column for separation. The analysis showed that WTCR (mesh 14-20) has great sorption capability for OTC, with a removal percentage of 60% - 95% depending on its initial concentration. A 48 hours WTCR stability test was performed at pH 1.5, 3.0 and 7.0 using an Inductive Coupled Plasma-Mass Spectrometry (ICP-MS) in order to determine the release of metals and their concentrations from the crumb rubber into the aqueous solution. The stability test showed that chromium and zinc were the most released. Also the adsorption behavior of Oxytetracycline onto carbon black in aqueous solution was also evaluated, as carbon black is one of the main components present in the crumb rubber. The results demonstrated that it has nearly 100% removal of OTC and shorter equilibrium times when compared with WTCR. We also evaluated the sorption behavior of oxytetracycline using WTCR mesh 30 (<0.67 mm of diameter). Our results show that WTCR mesh 30 has better sorption capabilities than WTCR 14-20.

## Resumen:

Actualmente dos de las formas más comunes de contaminación son: (a) exceso de compuestos antimicrobianos (oxitetraciclina, OTC); y (b) desperdicios de neumáticos usados.

Nuestra investigación evaluó el comportamiento de sorción de la miga de neumático en solución acuosa. Previamente se ha evidenciado que la miga de neumático tiene un gran potencial para la absorción de los compuestos antimicrobianos. Inicialmente realizamos un barrido, utilizando espectrofotómetro UV-Vis, y se determinó que la longitud de onda de absorción máxima fué de 353 nm. Las muestras de OTC de 25 a 150 mg/L (ppm) a pH neutro, se pusieron en contacto con 10 g/L de miga de neumático malla 14-20 (2.45 mm de diámetro), durante 84 horas. Las muestras fueron colectadas a diferentes intervalos y analizadas por Cromatografía Líquida de Alta Eficiencia con un Detector de Arreglo de Diodos (HPLC-DAD), utilizando una columna de separación Eclipse XDB-C8. El análisis mostró que la miga de neumático (malla 14-20), posee la capacidad de absorción ideal para OTC, con un porcentaje de remoción de 60% - 95%, dependiendo de su concentración inicial. La miga de neumático fué sometida a una prueba de estabilidad durante 48 horas, (pH 1.5, 3.0 y 7.0) utilizando un ICP-MS, (por sus siglas en inglés), para determinar qué metales se encuentran presentes en la miga de neumático y cuales son sus concentraciones. La prueba de estabilidad arrojó la presencia de cromo y zinc en altas concentraciones. Fué evaluado el comportamiento de adsorción de la OTC en el carbón negro en solución acuosa. Al ser el carbón negro uno de los principales componentes presentes en la miga de neumáticos, los resultados demuestran que éste posee casi un 100% de remoción de OTC y tiempos de equilibrio más cortos. Evaluamos y analizamos el comportamiento de absorción de la OTC con malla WTCR 30 (<0,67 mm de diámetro). Nuestros resultados muestran que la malla WTCR 30 tiene una mejor capacidad de absorción de WTCR 14-20.

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To God, my family and friends.

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## ***1. INTRODUCTION***

Nowadays our world experiences serious environmental problems of which one is caused by the massive use of antimicrobials such as Tetracyclines (e.g. Oxytetracycline). According to the literature 8.5 million grams of antimicrobials are used in the US for agricultural purposes annually <sup>(1)</sup>. Even though these antimicrobials accelerate growth and allows animals to be brought faster and at a lower cost to the market, up to 90% of the antimicrobials fed to animals are excreted in their active form, thus making the manure an agent that alters the soil microbial composition. This results in an accelerated evolution of antimicrobial-resistant bacteria as well as a transfer of resistance to other bacteria in the environment <sup>(2)</sup>. Another environmental problem is the huge stockpiles of rubber from discarded car tires. It's been estimated that around 10 million tires are discarded every year worldwide. These tires not only represent a serious fire hazard, but also becomes a breeding ground for disease carrying animals. Puerto Rico is not exempt of both problems.

The approach of this research is based on the presence of the main components in crumb rubber from tires such as sulfur, carbon black, polymeric styrene-butadiene and zinc oxide. These components have the potential to interact and have shown a sorption capability for organic species, heavy metal ions and antimicrobial compounds from aqueous solutions <sup>(3, 4, and 5)</sup>. In order to address the critical issues regarding the recycling of used rubber tires and the removal of antimicrobial contaminants from water bodies this project focused on the use of WTCR as a sorbent for the removal of OTC from aqueous solutions, thus working on both of the stated problems at the same time. The WTCR has a complex matrix in which carbon black and polymeric styrene butadiene are its main components. It is expected that the non-polar organic compounds interact with the non-polar matrix of the rubber through Van der Waals interactions.

In contrast, the polar compounds expected to interact by means of dipole-dipole interactions and hydrogen bonding. An important fact worth mentioning in the sorption process is the vulcanized rubber whose molecular structure contains a large number of cross-link. Based on this we can assume that the greater the amount of cross-links in the rubber, the shorter the average length and the lower the rate of diffusion of the compound. For a faster diffusion of organic molecules into the rubber they must have a lower molecular weight. There is a proposed mechanism for the sorption of the Tetracyclines (TCs) in the rubber matrix, which consists in the formation of chelates within the rubber. Van der Waals interactions are expected between the TCs antimicrobials and the carbon black contained in the crumb rubber.

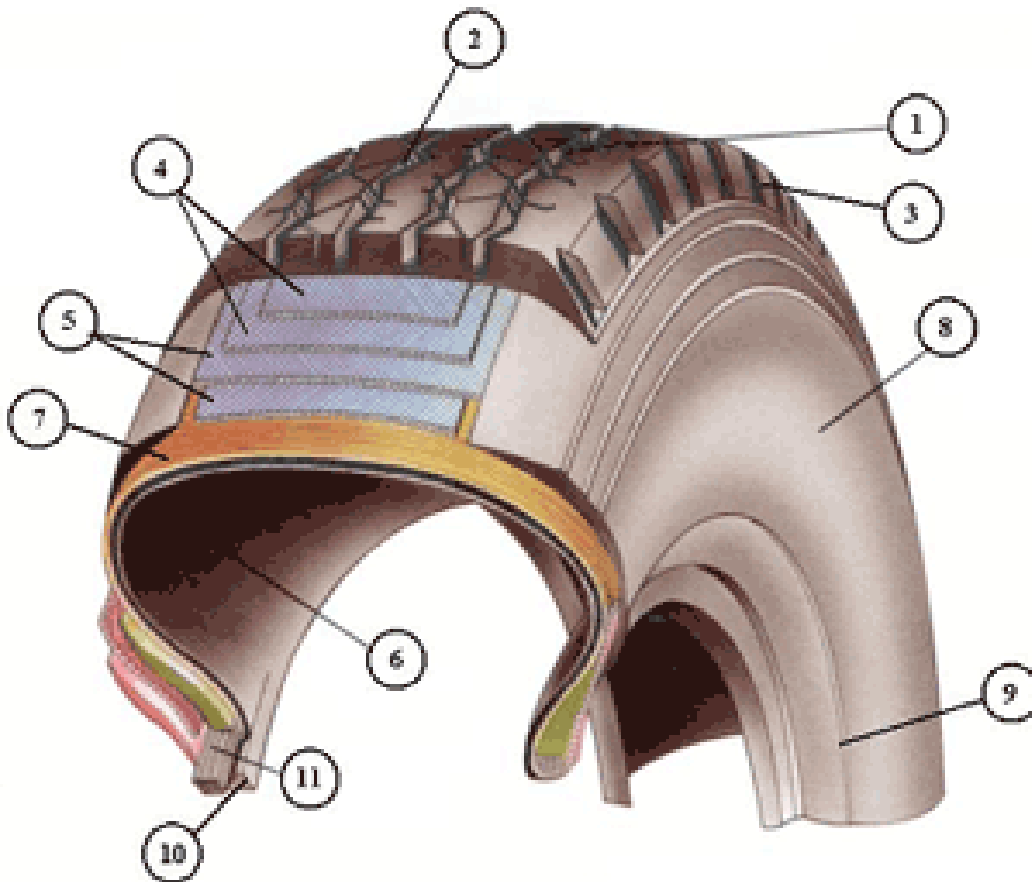
The sorbent material for this research, already cleaned and downsized to different particles sizes, was kindly provided by Rubber Recycling and Manufacturing Corp., REMA in Caguas, Puerto Rico. Evidently the applicability of crumb rubber as a sorbent will expand the recycling options for this material towards the treatment of polluted water bodies and the remediation of contaminated soils, where eventual superficial or underground water streams could have mobilized antimicrobials into the aqueous phase.

## ***2. LITERARY REVIEW***

### ***2.1 Crumb Rubber from Waste Tires***

#### ***2.1.1 Structure - Tire Components and Definitions of Technical Terms***

Tires are composed of various materials, these range from various types of steel to rubber compounds (as the main components in a tire structure). In this section we aimed to describe the technical terms used to identify tire components, which are shown in figure 1 and described in table 1 below:



***Figure 1: Components of a tire <sup>(6)</sup>***

**Table 1: Tire Components <sup>(6)</sup>**

Tread (1)	Part of a pneumatic-tire that is designed to come into contact with the ground.
Tread groove (2)	The space between the adjacent ribs or blocks in the tread pattern.
Sidewall (3)	Part of a pneumatic-tire between the tread and the area designed to be covered by the rim flange.
Ply (4, 5)	A layer of "rubber" coated parallel cords. In the radial tire, it has the purpose of stabilizing the tire.
Cord (6)	The strands forming the fabric of the plies in the pneumatic-tire.
Carcass (7)	Structural part of a pneumatic-tire other than the tread and outermost "rubber" of the sidewalls which, when inflated, supports the load.
Section width (8)	The linear distance between the outside of the sidewalls of an inflated pneumatic-tire, when fitted to the specified measuring rim, but excluding elevations due to labeling (marking), decoration or protective bands or ribs.
Belt (9)	Applies to a radial ply or bias belted tire and means a layer or layers of material or materials underneath the tread, laid substantially in the direction of the centre line of the tread to restrict the carcass in a circumferential direction.
Bead (10)	Means the part of a pneumatic tire that is of such shape and structure as to fit the rim and hold the tire onto it.
Chafer (11)	Means material in the bead area to protect the carcass against chafing or abrasion by the wheel rim.



### 2.1.2 Tire Composition

The components of a new tire are shown in Table 2, and the materials used in its manufacturing are shown in Table 3.

*Table 2: Main components of WTCR <sup>(7)</sup>*

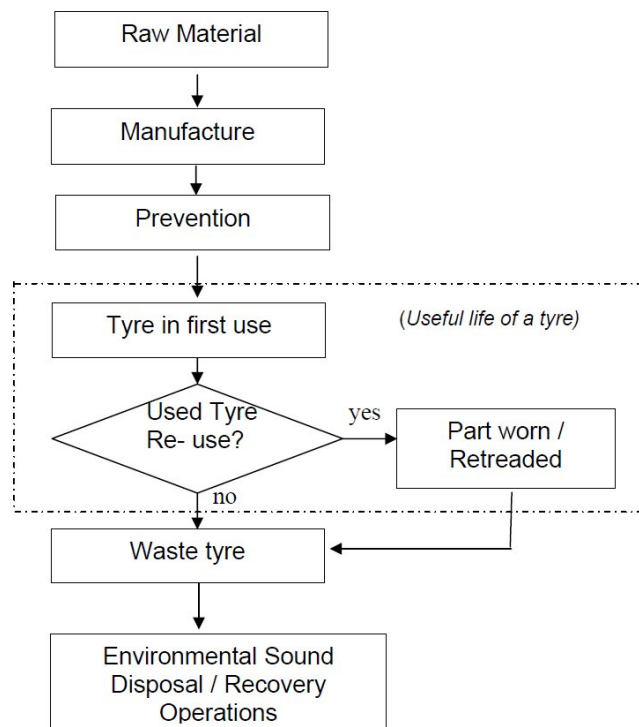
WTCR Composition	
Compound	% W/W
Rubber	40 ~ 60
Carbon Black	20 ~ 30
Metal	10 ~ 25
Zinc Oxide	2 ~ 3
Sulfur	1 ~ 3
Stearic Acid	1 ~ 2
Extender and Process Oil	2 ~ 20

**Table 3: Materials used in the Manufacture of Tires <sup>(6)</sup>**

Material	Source	Application
Natural Rubber	Natural rubber is predominantly obtained from the sap of the <i>Hevea brasiliensis</i> tree.	Generally Natural rubber currently accounts for about 30% to 40% of the total elastomeric part in a car tyre and 60% to 80% of a truck tyre.
Synthetic Rubber	All synthetic rubbers are made from petrochemicals	Generally synthetic rubber accounts for about 60% to 70% of the total elastomeric part in a car tyre and about 20% to 40% of a truck tyre.
Steel cord and bead wire including the coating materials and activators, brass /tin/zinc.	The steel is premium grade and is only manufactured in a few plants around the world due to its high quality requirements.	Steel is used to provide rigidity and strength to the tyres.
Reinforcing fabrics	Polyester, rayon or nylon	Used for structural strength and of the carcasses of car tyres.
Carbon black, amorphous silica	Carbon black is derived from oil stock. Amorphous silica is obtained from silicium mineral and sodium carbonate. It may have natural or synthetic origin.	Carbon black and silica provide durability and resistance against wear and tear.
Zinc oxide	Zinc is a mined mineral or also derived from recycled zinc, which then undergoes a production process to produce zincoxide.	Zinc oxide is added essentially as vulcanization activator. After vulcanization it is present as bound zinc in tyres.
Sulphur (including compounds)	A mined mineral or extracted from gas or oil.	Main actor or vulcanization.
Other additives and solvents age resistors, processing aids, accelerators, vulcanizing agents, softeners and fillers	Synthetic or natural source.	The other additives are used in the various rubber compounds to modify handling manufacturing and end-product properties.
Recycled rubber	Recovered from used tyres or other rubber products.	Used in some rubber compounds in the manufacture of 'new' rubber products and retread materials.

### 2.1.3. Tire Production Cycle

There are various stages when making a new tire, from when raw material is acquired to its disposal, are shown in Figure 2.



*\*Tyre = Tire*

**Figure 2: Tire Production Stages** <sup>(6)</sup>

### 2.1.4. Recycled Tires

Some countries around the world (e.g. UK) are allowing the retail of used tires. However, it is worth noting that there are risks involved when purchasing a used tires, since the manner in which the tire were used is totally unknown. Some of these tires could have come from vehicles involved in accidents and/or damaged by potholes and obstacles.

Partly worn-out tires can be reused without further treatment. Sources of used tires include:

- a. Tires fitted to second-hand vehicles that are sold and from vehicles that are scrapped.
- b. Old tires that can be used for less demanding applications.
- c. Tires that are exchanged for reasons other than that the tires have reached the end of their life, such as fitting a set of high performance tires or different wheels.

The United Kingdom has legislations under its Motor Vehicle Tires Safety Regulations of 1994, which regulates the sales and distribution of used tires. The requirement for selling and distributing these tires are:

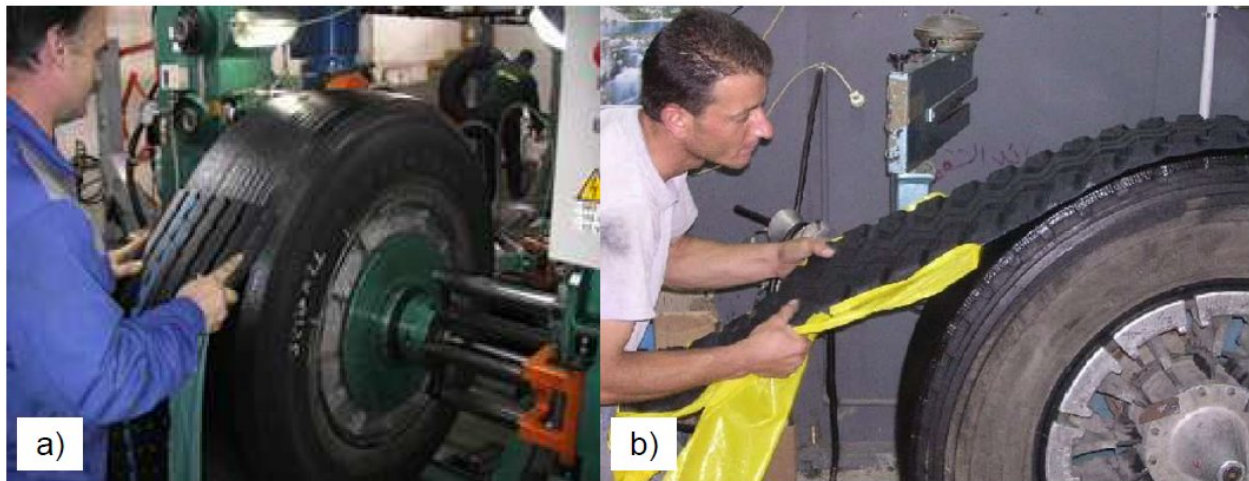
1. The tire may not have any cut in excess of 25 mm or 10% in its section width.
2. The tire may not have any external lump, bulge or tear caused by the separation or failure of its structure.
3. The tire may not have any of the ply or cord exposed.
4. When inflated to the highest pressure at which it is designed to operate, the tire may not exhibit any of the defects described above.
5. The base of any groove that showed in the original tread pattern must be clearly visible.
6. The grooves of the original tread pattern must be at least 2 mm deep.

According with the “Revised Technical Guidelines on Environmentally Sound Management of Used Tire of 2010”, there are studies being made to equip tires with electronic chips called Radio Frequency Identification Device (RFID) that record information about their conditions of use. If their efficiency can be proven, RFID may provide a means to identify the appropriate conditions for re-using used tires.

### 2.1.5. Retreaded Tires

The term “retreading” means to replace the wearing surface of the tire. They are three different types of processes:

1. *Top-capped tires*: are those in which the tread is removed and replaced with a new arranged one.
2. *Re-capped tires*: have their tread removed; but in this case the new tread used is larger than in the re-topped tire, as it covers part of the tire’s sidewalls
3. *Bead to bead tires*: are those in which the tread is removed and the new one goes from the one side to the other, covering all of the lower part of the tire and cover the sidewalls with a rubber layer.



**Figure 3: Retreading process** (*nsbtire.com* , *adamteva.org.it*)

In accordance to United Nations Economic Commission for Europe (UNECE) regulations 108 and 109 <sup>(8)</sup> established the requirements for approval of the production of retreaded tires, which are:

1. Automobile tires may be retreaded only once, while
2. Trucks tires may be retreaded up four times
3. Aircrafts tires up to ten times.

In order to meet safety standards, tire retreading should only be carried out by qualified companies and tires should be certified to guarantee safety and quality standards for consumers.

Environmental impacts of retreading tires are generally positive. The impacts from retreading should be contrasted with impacts of manufacturing new tires. Retreading a tire consumes considerably less material and energy than that required for a new tire, with a proportional decrease in other impacts. A number of authors have published data in broad terms about the energy and material savings from retreading. Retreading utilizes a significant proportion of the rubber and all the fabric and steel in a tire. The processing energy is reported to be lower than for a new tire though the actual reduction varies depending on the type of retreading (whether hot or cold or remoulding). The estimates available for tires indicate that retreading has significant potential to reduce overall energy and greenhouse emissions, as well as reduce the quantity of waste tires that are produced <sup>(6)</sup>.

Tire retreading is definitively beneficial for the environment since it minimizes the generation of waste and increases the useful life of tires, thereby postponing their final disposal.

The main environmental impacts caused by the process of tire retreading are shown in Table 4.

**Table 4: Environmental Impacts of Retreading <sup>(6)</sup>**

Energy and material use	<p>As retreading extends the life of a tyre and utilizes much of the original materials and structure, the net result is a decrease in materials and energy used in comparison with new tyres.</p> <p>The energy used to retread a tyre is approximately 400MJ compared to 970 MJ for manufacturing a new tyre.</p>
Air emissions	The primary areas of concern are volatile organic compounds (VOCs) from solvents, bonding agents and rubber compounds during vulcanization. Odor may also be an issue in some areas.
Solid wastes	The process generates significant wastes. The rubber removed from used tyres before retreading is generally sold as rubber crumbs for other purposes.

Besides retreading, waste tires can be recovered by being cut, shredded or grounded and used in several applications, such as in footwear, sports surfaces, carpets, etc. Waste tires can also be used as Tire-Derived Fuel (TDF) for energy recovery.



### 2.1.6. Potential risks to health and the environment

The constituents of tires do not make them exhibit hazardous properties. However, if they are not properly managed and disposed, tires will become a certain risks for public health and the environment. The problem with waste tires is that they are not biodegradable and take up a lot of physical space.



*Figure 4: Close-up look at a Tire Landfill* ([blog.timberland.com](http://blog.timberland.com))

#### 2.1.6.1. Risks to Public Health

Waste tires are ideal sites for rodents and also breeding sites for mosquitoes that transmit dengue among other diseases. The latter being of great relevance in for tropical and subtropical regions. The round shape of tires and their impermeability enable them to hold water for long periods of time, turning them into perfect sites for the development of mosquito larva. Tires facilitate the spread of two species of mosquitoes, *Aedes aegypti* and *Aedes albopictus*. These are the principal infectious agents of dengue and yellow fever diseases that afflict millions of people in tropical regions <sup>(9)</sup>. In temperate regions, other species such as *Aedes triseriatus* and *Aedes*



*atropalpus* are more predominant. The movement of used tires not only spreads mosquitoes, but also contributes to the introduction of non-native species, which are often more difficult to control, increasing the risk of disease. The rapid geographic spreading of these mosquitoes can be largely attributed to the international trade of used tires <sup>(6)</sup>.



***Figure 5: Discarded tire serving as mosquitoes breeding sites*** ([dreamstime.com](http://dreamstime.com))

The Tiger mosquito (*Aedes albopictus*) was first introduced into the south-east U.S. in the late 1980s thanks to the importation of used tires from Asia. It spread rapidly along north-south transportation routes aided by the movement of goods and people, and has displaced native species of mosquitoes in some areas. No human case of viral transmission due to *Aedes albopictus* has been documented in the U.S. or Canada to date. The Tiger mosquito has been found as far north as Chicago but it does not survive the winters in northern U.S. nor has it ever been identified in Canada <sup>(10)</sup>.

This evidence demonstrates conclusively that the accumulation of used and waste tires, as well as their transport, poses a genuine risk of diseases that are transmitted by mosquitoes. Companies involved in transport and management should be aware of this issue and handle tires in such a way that the spreading of such diseases is prevented or reduced.

The World Health Organization (WHO) <sup>(11)</sup> indicates that the most effective means of vector control is environmental management. They include planning, organization, carrying out and monitoring activities for the modification of environmental factors while trying to prevent or reduce the propagation of these. One of the important factors influencing these contacts is the fact that in urban area's waste is not collected often but abandoned in the vicinity of housing areas. Moreover, used tires are often used by the population for various purposes, including use to plant flowers; provide ballast on roofs and manufacturing toys for children. Filling, covering or collecting tires for recycling or disposal are mentioned as measures to control pests in these cases. This stresses the importance of awareness raising and a good functioning collection and management system for tires.

#### **2.1.6.2: Environmental Risks**

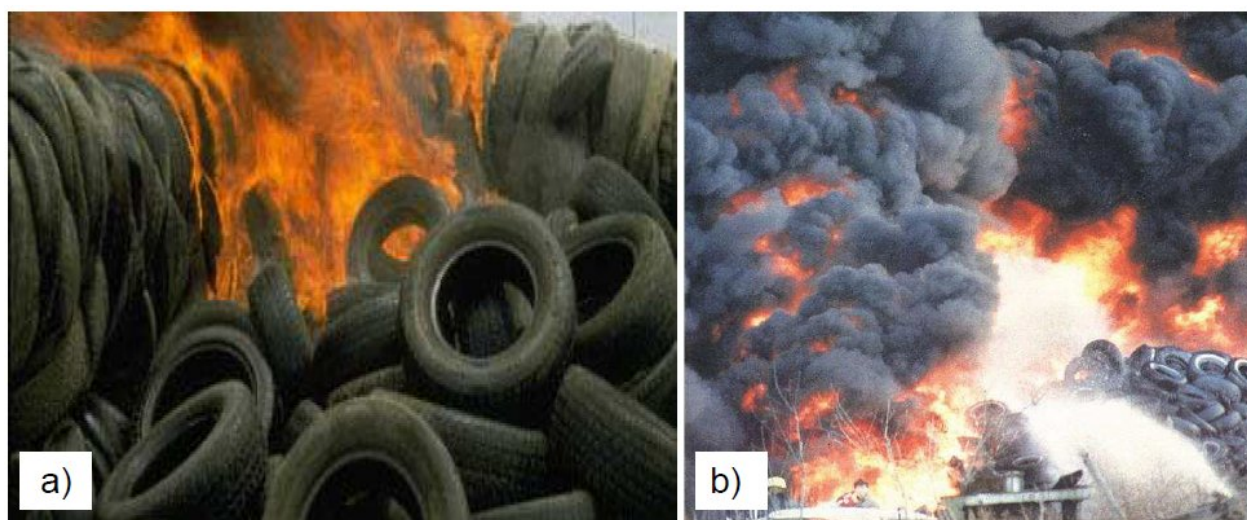
##### **2.1.6.2.1: Ecotoxicity**

In 2003, tests conducted by Birkholz <sup>(12)</sup> in California using rubber crumbs taken from a site where the tire had been disposed showed toxicity to: bacteria, invertebrates, fish and green algae. After three months, new samples were tested, demonstrating a 59% reduction in the toxicity detected in previous tests.

In addition to the acute or short-term toxicity addressed above, long-term studies should also be taken into account. Long-term investigations point to the fact that certain types of tires (e.g. high aromatic oil-containing tires), under certain conditions may leach significant amounts

of PAH into the aquatic environment <sup>(13)</sup>, thus influencing the population dynamics of wood frogs <sup>(14)</sup>.

Other studies reveal that leaching of heavy metals and organics chemicals such as Phthalates and PAH, from recycled car tires as infill in artificial turf systems stays well within the Dutch limit values for soil and surface water quality <sup>(6)</sup>. Leaching of zinc is an exception but is not expected to exceed limits values within 10 years.



**Figure 6: Burning tires represents another ecotoxicity problem** ([tracypress.com](http://tracypress.com))

#### **2.1.6.2.2: Leaching**

Water generated by tire leachate is bound to contaminate both soil and surface water and groundwater. Based on specialized literature and their own experience, the Ministry of the Environment of New Zealand <sup>(14)</sup> pointed out several factors that may affect the rate of leaching and/or the concentration of tire leachate compounds in soil, surface water and groundwater. Recent studies looked into the environmental aspects of the application of tire granulates as filler in artificial sportsfields <sup>(15)</sup>.

Analytically detectable trace compounds are dissolved from the surface and from the polymer matrix of the crumb rubber in a concentration which falls over time. The concentrations of the measured individual substances, the Dissolved Organic Carbon and the organic nitrogen decrease very rapidly initially, subsequently slowing down to a minimum in a time-dependent, substance-specific manner both in the lysimeter trials and the eluate tests. Towards the end of the trial period, after a year, values have already fallen below the limit of determination for most of the individual substances <sup>(16)</sup>.

The very low PAH concentrations from the granules were found at an identical level in the blank sample (gravel layer without surface); they correspond to ambient (ubiquitous) contamination levels <sup>(19)</sup>. Leaching of heavy metals and organics chemicals such as Phthalates and PAHs, from recycled car tires as infill in artificial turf systems stays well within the Dutch limit values for soil and surface water quality. Leaching of zinc is an exception but is not expected to exceed limits values within 10 years <sup>(20)</sup>.

According to a 2007 research, the physicochemical parameters of crumb rubber were identified and their ecotoxicological tests show that water passing through artificial turf using as filling crumb rubber is not likely to affect water resources in the short and medium term <sup>(19)</sup>.

A research done by the European Association of the Rubber Industry suggests that the effects due to leaching of chemicals from used tires in generally are negligible. A further study looked at literature addressing the potential of chemicals to leach from used tires and concluded that the impact of the application of used tires in subsoil of roads or surface waters under neutral environmental conditions has negligible effects on the ground and surface water quality and the aquatic environment <sup>(21)</sup>.

Based on the cited previous works we can conclude that crumb rubber can be used without being preoccupied of significant leaching of organic compounds or metals ions.

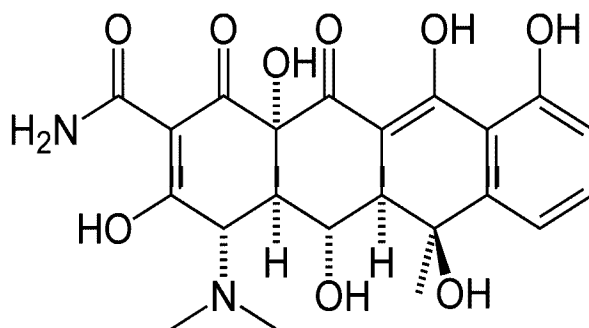
#### **2.1.6.2.3. Puerto Rico scenario**

In Puerto Rico over 4 million tires are annually discarded. This represents near to 15,000 tires per day, which makes the problem of solid waste management to become even more difficult to control. Approximately 800,000 tires are reused each year, the remainder is landfilled, stockpiled or illegally dump <sup>(22)</sup>. This in turn becomes a potentially serious problem not only in terms of safety but also a health hazard. Like in other locations around the world, improperly disposed tires serve as breeding grounds for diseases carrying mosquitoes and rodents; while in the safety area the stockpiles of tires are a serious fire hazard and, once ignited, the tires can burn out of control for months, producing acrid black smoke and hazardous oily residue. Thousands illegally dumped tires are found in streams, river and roadside throughout the Island. This mismanagement represents a significant waste of resources.

Based on the facts mentioned above (the still growing generation rate of new tires and the stricter controls onto used-tires disposal) the search of a new option to re-use this waste material becomes indispensable, not only for PR but also for the US.

## 2.2 Oxytetracycline

Oxytetracycline (OTC) 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<sup>(26)</sup>.



*Figure 7: Oxytetracycline molecular structure*

### 2.2.1. History:

It was first found by Finlay et al<sup>(26)</sup> near Pfizer laboratories in a soil sample yielding actinomycete, streptomyces rimosus. 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<sup>(27)</sup>.

### 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 <sup>(28)</sup>.

### **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 (e.g. pneumonia) <sup>(28)</sup>. Oxytetracycline is used to treat acne, due to its activity against the bacteria on the skin that causes acne (*Propionibacterium acnes*). It is used to treat flare-ups of chronic bronchitis, due to its activity against the bacteria usually responsible, *Haemophilus influenza* <sup>(28)</sup>. Oxytetracycline may also be used to treat other rarer infections, such as those caused by a group of micro-organisms called rickettsiae (e.g. 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 to test for its bactericidal activity <sup>(28)</sup>.

### **2.2.4. Veterinary Indications:**

Oxytetracycline is indicated in the treatment of respiratory infections: bronchopneumonia, shipping fever (pasteurellosis), atrophic rhinitis & enzootic pneumonia in pigs, mixed infections & necrobacillosis. It is also used to treat gastro intestinal infections caused by *E. coli*, salmonella and anaerobes; urinary infections, (endo) metritis, acute mastitis, septicemia, infectious polyarthritis, leptospirosis, foot rot, erysipelas, infected wounds & skin infections (exudative epidermitis in piglets). It is also used for bacterial infections secondary to viral ones, such as Anaplasmosis <sup>(28)</sup>.

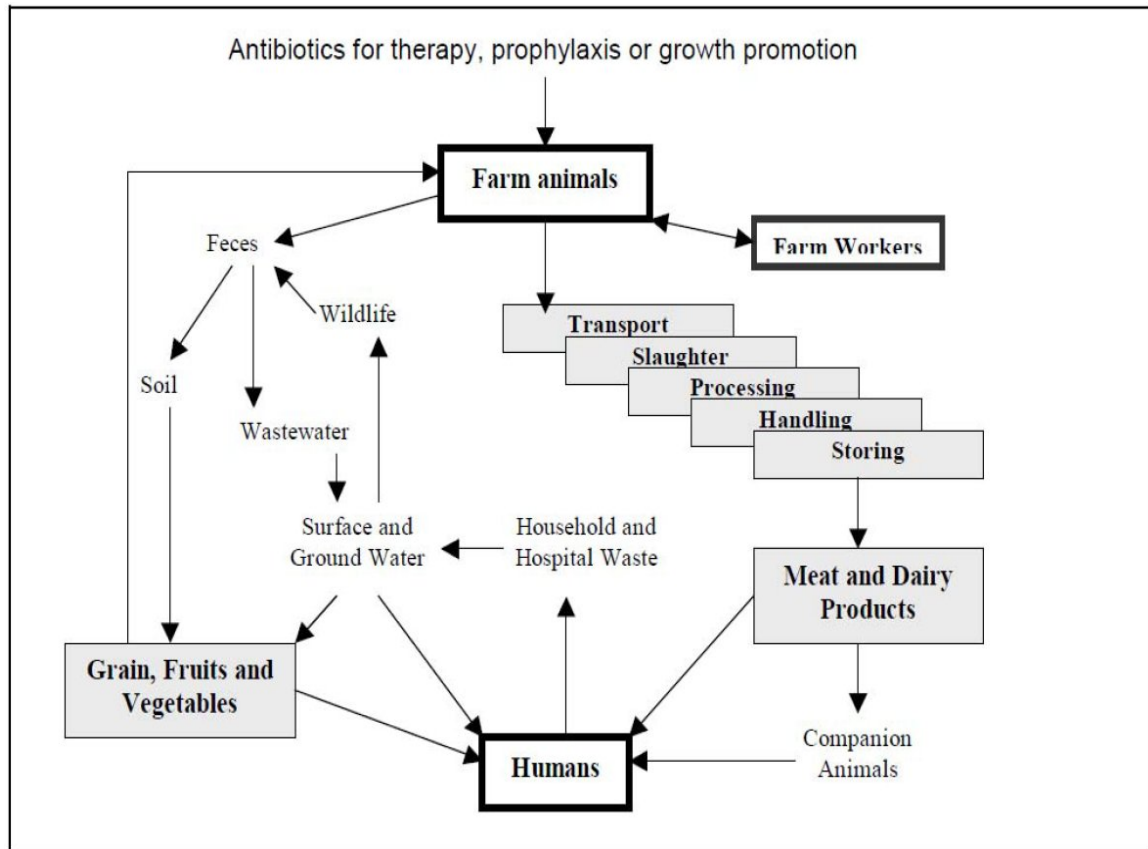
### **2.2.5. Antibiotics in Animal Husbandry:**

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:

1. 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 sub clinical stage of the disease <sup>(29)</sup>.
2. 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 sub clinical 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 <sup>(29)</sup>.
3. Sub therapeutic 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 <sup>(29)</sup>.

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.





**Figure 8: Movement of antibiotics from animals to humans** <sup>(30)</sup>

### 2.2.6. 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.

1. Improved food safety and quality can be observed due to healthier animals in general and thus due to reduced pathogen contaminations of animal products <sup>(29, 31)</sup>.
2. Cost reduction due to lower loss rates and productivity gains, e.g. enhanced growth in fattening animals <sup>(32)</sup>.

3. 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 <sup>(29, 33)</sup>.

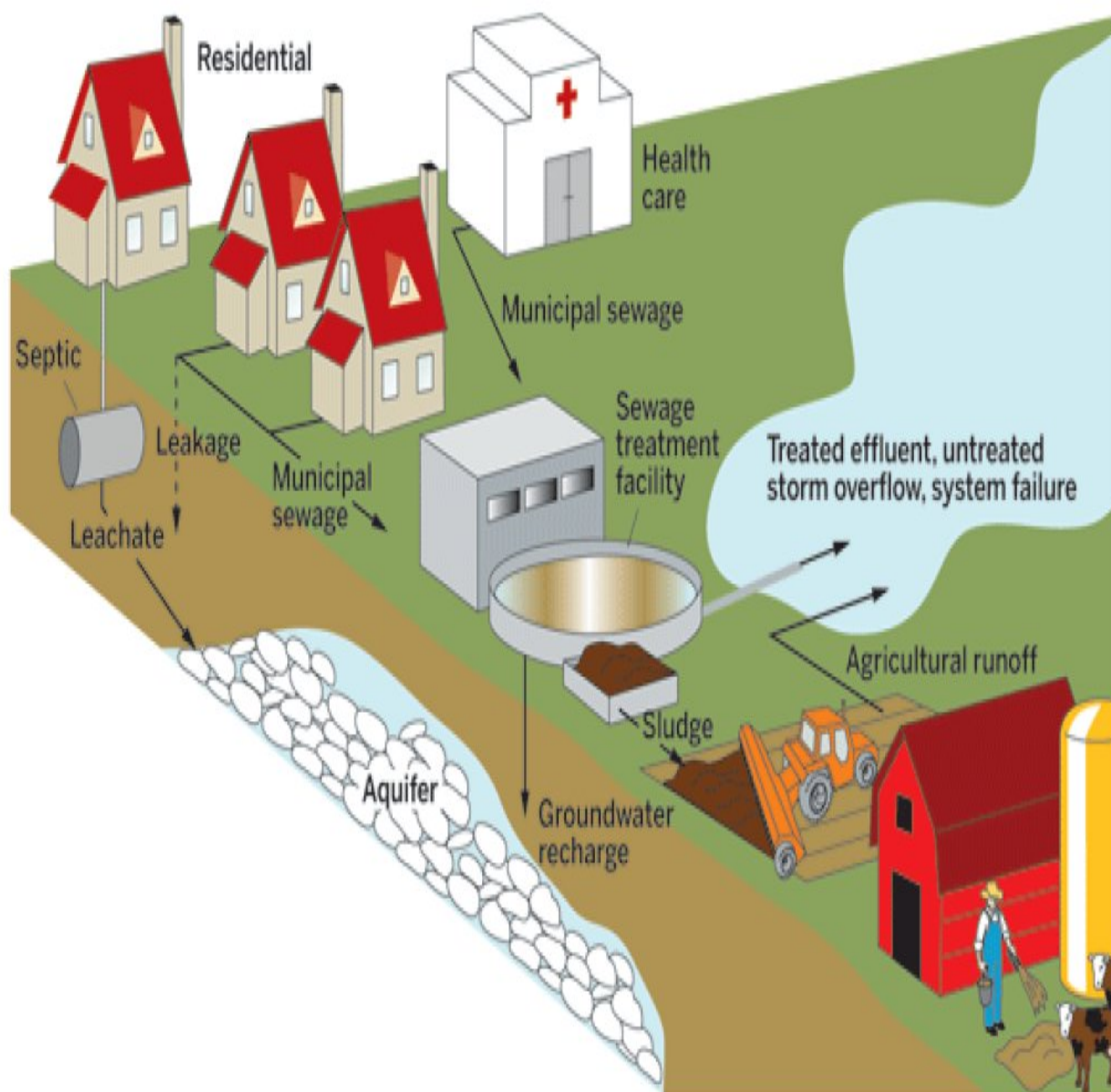
Although critical voices have been raised since the very beginning of antibiotic use in agriculture, it has only been in the past decade that the risks of antibiotics have received considerable public attention <sup>(34)</sup>. Possible hazards relate to the furthering of zoonotic pathogens, which can 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 <sup>(29, 35)</sup>.

#### **2.2.7. Antibiotics in the Aquatic & Terrestrial Environment:**

People all over the world are prescribed millions of doses of antibiotic drugs, including those used for Livestock. 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, these compounds start to appear in waste water, soil & ground water.

It was found that humans consume 235 million doses of antibiotics each year. In 2004 Twenty one (21) million pounds of antibiotics were administered by livestock & poultry producers <sup>(36)</sup>. 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 <sup>(29)</sup>. Out of 139 streams in 30 states, it was found that about 80% contained trace amounts of these 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 <sup>(37)</sup>.



**Figure 9: Flow of antibiotics and their metabolites into sewage systems <sup>(38)</sup>**

### **2.2.7.1. Puerto Rico scenario:**

Tissue samples from cattle, calf and swine containing huge levels of agricultural residues have been found in PR through 1999 – 2001. The rate of violations was mostly confined to cattle with 70 violations (antibiotics - 68; sulfonamide - 1) and was due to the presence of excessive amounts of antibiotics in the animal tissues, as stated in the Food Safety and Antimicrobial Residues in Food Animals (information available at:

<http://www.fsis.usda.gov/OPPDE/animalprod/Presentation/Residue>). It is evident that the presence of antimicrobials in PR agricultural soils and water bodies can't be ruled out.

### **2.2.8. 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 <sup>(27, 29)</sup>.

- 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 usage 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 of which 25 million pounds 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, quaternary 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 sub therapeutic 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 from animals sources. A popular theory is that vancomycin resistant strains of the bacterium *Enterococcus* (VRE), a major cause of post surgical infections, have arisen in Europe due to the use of the antibiotic avoparcin as an animal growth promoter <sup>(29)</sup>.

The use of oxytetracycline in aquaculture has been shown to cause a seasonal shift in bacterial species and is associated with increased antibiotic resistance <sup>(39)</sup>.

### **2.2.9 Bacterial Resistance on the Rise**

The World Health Organization (WHO) has recently identified antibiotic resistance as a major problem for public health. While overuse and inappropriate application in human medicine have been found to be probably the most significant sources of risk, the use of antibiotics in animal husbandry had also contributed to the problem.

However, both the complexity of the issue and the lack of data proved to be serious obstacles on the way to evaluating the possible risks from that source of resistance <sup>(40, 41)</sup>. There have been serious concerns about the massive amounts of antibiotics used to treat livestock may be creating antibiotic resistant microbes. According to W. Kolping <sup>(42)</sup> over 500 millions tons of livestock pharmaceutical waste is generated annually. W. Kolpin also points out that livestock manure is chockfull of antibiotics. Farmers use the sludge generated by sewage treatment plants as a fertilizer and a source of nutrients for crops, but this material also contains excreted medications <sup>(43)</sup>. Experts claim that bacterial resistance will make possible infectious disease epidemics more potent and deadly than any have been experienced in human history.

Due to global travel and widespread commerce, drug resistance is expected to spread steadily to all parts of the world. 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 antibiotics alters the environment in favor of those bacteria that are resistant to it <sup>(44)</sup>.

### **2.3 Adsorption Isotherms**

Adsorption is the process where the adsorbate (OTC) is attached to the surface of the adsorbent (crumb rubber). Typically this process is used to separate an adsorbate from a matrix, such as a solvent. Adsorption must not be mistaken for absorption, which is a process where a solute enters to the bulk phase of the adsorbent. Sorption is the process where adsorption and absorption occur at the same time or one process can not be simply distinguished from another. The efficiency of the adsorption process depends on the surface characteristics of the material such as porosity. With a higher porosity, the material will have a higher surface area to volume ratio and as a consequence its capacity of adsorption will increase. For this process, a dynamic equilibrium can be reached when the number of molecules or atoms leaving the surface is the same as the number of molecules adsorbed at the adsorbent surface <sup>(4)</sup>.

At equilibrium, an adsorption isotherm can be constructed as a relation of the concentration of the adsorbate in the surface and in the solution at a given constant temperature.

The isotherms used were: Linear, Freundlich, and Langmuir isotherms.

### 2.3.1 Linear Isotherm

The linear isotherm is described by the following equation:

$$C_s = K_d C_f \quad (2.3.1)$$

Where:  $C_s$  = concentration of adsorbate in solid phase at equilibrium (mg/g);

$C_f$  = concentration of the solute on fluid phase at equilibrium (mg/L);

$K_d$  = distribution equilibrium coefficient (L/g).

The information that can be obtained from the graph  $C_s$  vs.  $C_f$  is the value of  $K_d$  from the slope of the linear curve. A greater value of the constant  $K_d$ , the faster the equilibrium is achieved. The linear isotherm is related to the Freundlich when the parameter  $n$  of the Freundlich isotherms is one, then the Freundlich isotherm can be simplified to a linear one.

### 2.3.2 Freundlich Isotherm

This isotherm predicts that, when the temperature of the system rises, the equilibrium is reached slower, and as consequence more concentration of sorbent is required to achieve the maximum adsorption capacity. This isotherm is applied to surfaces that are heterogeneous and for multilayer adsorption. The non linear form of the Freundlich isotherms is described mathematically with the following equations:

$$q_e = k \frac{C_e}{1} \quad (2.3.2 \text{ a})$$

Rearranging the equation 2.3.2.a applying the natural logarithm to both sides, results in the linear Freundlich equation:



$$\ln q_e = \ln k + \frac{1}{n} \ln C_e \quad (3, 45) \quad (2.3.2.b)$$

Where:  $q_e$  = solute concentration adsorbed at equilibrium (mg/g);

$C_e$  = concentration of adsorbate in the solution at equilibrium (mg/L);

$k$  = Freundlich isotherm parameter (mg/g), that represents the relative adsorption capacity of the sorbent.

$n$  = Freundlich parameter (g/L). The reciprocal value ( $1/n$ ), gives a relationship between the intensity of the adsorption between the adsorbate and the sorbent studied. Meaning when  $1/n$  is greater, the stronger is the adsorbate-sorbent surface.

### 2.3.3 Langmuir Isotherm

This isotherm is based on four basic assumptions, which apply for systems that form monolayers of adsorbate. The assumptions are listed below:

1. The solid surface is uniform and, as consequence, all sites available for adsorption are equivalents.
2. A dynamic equilibrium exists between the molecules leaving and those entering the surface of the adsorbate, at constant temperature.
3. Adsorbate molecules are constantly colliding with the surface, until a vacant site is reached and a bond is formed.
4. Once the adsorbate is adsorbed, it remains localized.

The Langmuir isotherm describes how a monolayer coverage of the adsorbate changes with variation of the adsorbate concentration. The eq. 2.3.3.a describes the Langmuir isotherm.

$$\frac{C_e}{q_e} = \frac{1}{Q_0 B} + \frac{C_e}{Q_0} \quad (3) \quad (2.3.3.a)$$

Where:  $C_e$  : concentration of solute in solution at equilibrium (mg/L)

$q_e$  : mg of solute (adsorbate) per gram of adsorbent at equilibrium (mg/g)

$Q_0$  : maximum adsorption capacity for a monolayer up to saturation (mg/g)

$B$  : Langmuir parameter (L/mg)

The Langmuir isotherm can be used to calculate a dimensionless parameter  $R$ , which describes the shape of the isotherm as the following Eq. 2.3.3.b shows:

$$R = \frac{1}{1 + (Q_0 C_i)} \quad (3) \quad (2.3.3.b)$$

Where the parameters are:

$Q_0$  = is the Langmuir parameter (mg/g);

$C_i$  = is the initial concentration of the solute (mg/L).

## 2.4 Previous Works

### 2.4.1 Crumb rubber as sorbent for organic and inorganic compounds in aqueous solutions.

Publications related to the specific use of crumb rubber for the removal of inorganic heavy metal ions from aqueous solutions are rare. The current literature mostly focused on batch experiments involving  $Cd^{+2}$ ,  $Pb^{+2}$ ,  $Hg^{+2}$ , without considering the influence of the speciation and oxidation state of the targeted inorganic species (factors that are highly related to the sorption behavior in most of the existent sorption systems).

Although the potential use of rubber particles to remove organic compounds and aqueous heavy metal ions ( $\text{Cd}^{+2}$ ,  $\text{Pb}^{+2}$  and  $\text{Hg}^{+2}$ ) has been preliminary evaluated <sup>(46-50)</sup>, recent literature lacks a comprehensive discussion of the involved mechanisms and explanations to the results observed. Knocke et al. <sup>(46)</sup> carried out studies to evaluate the sorption behavior of waste tire rubber for inorganic Hg from aqueous solution. In these experiments, ground samples of vulcanized tire rubber were used in batch sorption studies. The results showed that, pH was the most important factor, with an optimum range of 5.5-6.0. It was suggested that the diffusion of Hg through pores in the rubber sorbent was the rate-limiting step regarding final uptake. We suggest that using higher porosity crumb rubber will increase inorganic sorption <sup>(3, 4, 46)</sup>. Rowley, Husband and Cunningham <sup>(47)</sup> proposed tentative mechanisms for the adsorption of  $\text{Cd}^{+2}$ ,  $\text{Hg}^{+2}$  and  $\text{Pb}^{+2}$  onto shredded rubber. The suggested mechanisms were dependent on the type of metal ion; Hg and Cd uptake took place with a simultaneous release of native embedded Zn which suggested an ion exchange mechanism. On the other hand, Pb adsorption did not involve any Zn displacement.

Gunasekara and Donovan <sup>(48)</sup>, have shown that ground discarded tires can remove inorganic Hg ions from aqueous solutions. Two types of ground tire rubber, a mixture of natural and synthetic rubbers and a completely natural rubber were used in the form of black powder. The sorption of Hg was slower in comparison to organic compounds and it was found that its sorption affinity was found to be dependent of the concentration. Preliminary results showed that for 1 g of rubber powder in 100 ml of  $\text{Hg}^{+2}$  solutions, the complete sorption for all Hg occurred at concentrations below 4  $\mu\text{g/ml}$ . The kinetic data for the sorption behavior for Hg was considered insufficient to determine if the rubber particles had reached saturation after three days of contact. Although the effect of solution chemistry on the “binding” of Hg to rubber powder

was studied, the authors stated the need for a more detailed evaluation of the process rate and a better explanation of the involved sorption mechanisms in this system. Concerning the sorption capability of rubber for organic compounds, J. Y Kim et al. <sup>(50)</sup> conducted batch sorption tests for m-Xylene, ethyl benzene, toluene, trichloroethylene, 1, 1, 1-trichloroethane, chloroform and methylene chloride. The corresponding partition coefficients decreased from 977 L/kg for m-Xylene down to 13 L/kg for the methylene chloride. Although different experimental conditions were tested, like: ionic strength, pH, ground tire particle size and temperature, however no significant effect on the sorption behavior was observed. It was suggested that the organic compounds sorbed primarily onto tire rubber polymeric materials and partially into the carbon black component. Gunasekara et al. <sup>(48)</sup> also investigated the sorption of naphthalene and toluene by granular crumb rubber. The sorption of organic compounds was relatively fast and thirty minutes of contact time were enough to achieve an 80% of naphthalene removal from an initial concentration of 15 µg/l. Results showed that rubber particles had a greater affinity for naphthalene than toluene.

In 2006 Alamo-Nole used WTCR to remove Ethylbenzene (E), Toluene (T) and Xylene (X) from aqueous solutions. He used GC-MS to quantify ETX using various concentrations of crumb rubber at pH 1.5 and 6.0. Removal percentages of 99 %, 95 % and 77% were obtained for xylene, ethylbenzene and toluene respectively <sup>(3)</sup>.

Sanchez-Rivera (2007) studied the removal of heavy metal ions from aqueous solution using WTCR. In this research parameters such as pH, metal concentration, among others, were evaluated in order to optimize the sorption uptake conditions. Equilibrium between the metal and WTCR surface was reached faster at lower concentrations. Lead was the better sorbed metal,

followed by copper and finally cadmium with Langmuir constants of 1.744 mg/L, 1.245 mg/L, 0.532 mg/L respectively <sup>(4)</sup>.

#### **2.4.2 Sorption of antimicrobials from aqueous solutions.**

Current studies aim at understanding better the elimination of antibiotics during municipal wastewater treatment facilities including sorption onto sewage sludge and biotransformation. Giger et al. <sup>(51)</sup> described a study focused on the behavior of antibiotics in a wastewater treatment plant (WWTP), in which conventional activated sludge treatment followed by a sand filter was operated in parallel with a pilot-scale membrane bioreactor. The plant is located in Switzerland. However, recent results and the type of evaluated antimicrobials have not been published. Pedersen <sup>(52)</sup> and his colleagues have investigated the sorption of sulfamethazine (SMZ) and sulfamethoxazole (SMX) to smectitic clays and natural organic matter (NOM) in batch sorption tests. SAs sorption was strongly pH dependent with neutral species exhibiting maximal association with both specific clays and NOM. SMZ adsorption to specific clay depended on the type of exchangeable cations, whereas the type of exchangeable cations did not affect the extent of SMX adsorption.

D. Aga et al. <sup>(53)</sup> investigated the development of quantitative techniques to determine TCs and SAs in aqueous solutions, as well as the sorption behavior of TCs on activated clay in an effort to understand the interaction of this type of compound with soil particles. D. Aga's group also studied the factors that affected the degradation and mobility of veterinarian-used antibiotics in soil and water. These were needed to develop better management practices and techniques for the effective removal of these compounds from the municipal wastewater. Their research deals with the degradation of TCs in soil fertilized with manure, micro-biodegradation of TCs antibiotics, fate of SAs in the aquatic environment and the factors that affected the

mobility of tetracycline in soil. Analysis of the soil extracts showed the presence of non-degraded chlortetracycline and transformation products that include isochlortetracycline and anhydrochlortetracycline. Also, it was found that the presence of corn plants affected the degradation of chlortetracycline. They have also investigated the interactions of oxytetracycline, a popular TCs antibiotic, with model clay adsorbents as a function of suspension pH. The clay adsorbents used were the natural montmorillonite clay (SWy-2), Na-montmorillonite clay (Na-SWy-2), and hexadecyl trimethyl ammonium bromide-montmorillonite clay (organo-clay). The adsorption of oxytetracycline in the natural and sodium forms of montmorillonite clay decreases with increasing pH in the order  $\text{pH } 1.5 > \text{pH } 5.0 > \text{pH } 8.7 > \text{pH } 11.0$ , suggesting that cationic exchange interactions are dominant at lower pH values, when oxytetracycline has a net positive charge. On the other hand, the adsorption of oxytetracycline in organo-clay is highest at pH 5.0 due to strong hydrophobic interactions with organo-clay when oxytetracycline is zwitterionic.

Karthikeyan et al. <sup>(54)</sup> have investigated the sorption of ciprofloxacin (TC and fluoroquinolone) to Fe and Al hydroxides, well-known soils components, as well as by humic substances. The sorption behavior of ciprofloxacin took place with a strong pH dependence, minor differences between liquid scintillation counting (LSC) and HPLC measurements after both one day and one week, a maximum sorption of 72% unaffected by reaction time around neutral pH. In turn, tetracycline sorption exhibited mild-pH dependence and a significant difference between LSC and HPLC-determined removal levels. Elevated levels of dissolved Al in the presence of tetracycline indicated that the ligand promoted dissolution of Al hydroxide could have taken place.

To the extent of our knowledge, there are no systematic studies focusing on the use of sorbents, other than soils or activated clays, for the removal of TCs and SAs antibiotics from

aqueous solutions. Therefore, we believe that the suggestion of using crumb rubber, mainly based on its composition and behavior in presence of inorganic and organic species, could be a very important contribution to the study of alternative solvents to soils, particularly for SAs compounds which exhibit a very high mobility in aqueous solutions. Furthermore, to the best of our knowledge, there are no reports dealing with the desorption behavior of the granular crumb rubber. Our research project will become indispensable for two reasons: a) to determine the viability of regenerating the sorbent and reuse together with the recovery of the sorbed species, or b) to establish the physical and chemical stability of sorbed species in crumb rubber to make its life-term disposal a safe option.

#### **2.4.3 Tetracycline Adsorption from Aqueous Solutions.**

In 2006 Nieto-Zambrano <sup>(55)</sup> evaluated the adsorption behavior of tetracycline crumb rubber in aqueous solution. Tetracycline samples at pH 3.80 with exposition times up to 168 hours at room temperature were placed in contact with crumb rubber. The samples were removed at different time intervals and analyzed by UV-Vis spectrophotometer, liquid chromatography coupled to mass spectrometer (LCMS) and diode array detection. The results showed that the sorption of tetracycline (TC) was dependent on mesh size and presence of Zn in solution. TC removal for WTCR mesh 14–20 and mesh 30 were 48.22 % and 59.80 %, respectively. These results were compared with carbon black present in crumb rubber matrix at 10.97–45.60 %, w/w. TC was completely removed from aqueous solution after 24 hours when placed in contact with carbon black suggesting its involvement in the TC adsorption by crumb rubber.

### 3. EXPERIMENTAL SECTION

#### 3.1 Reagents and Materials

**Table 5: Reagents List**

Oxytetracycline HCl 95% from Sigma-Aldrich
Acetonitrile HPLC Grade 99.99% from Agros Organics
Distilled and Deionized Water (18.1 mΩ-cm)
Nitric Acid (HNO <sub>3</sub> ), Trace Metal Grade, ACS Certified
Carbon Black N330, Sid Richardson Carbon Company, CAS # 1333-86-4
Crumb Rubber, REMA Inc. (mesh 14-20 and 30)
Sodium Hydroxide (NaOH) Fisher Scientific, Analytical Grade
Argon Gas 99.998 % purity, from Linde
Cadmium Standard 1000 ppm
Copper Standard 1000 ppm
Zinc Standard 1000 ppm
Arsenic Standard 1000 ppm
Lead Standard 1000 ppm
Chromium Standard 1000 ppm

**Table 6: Materials List**

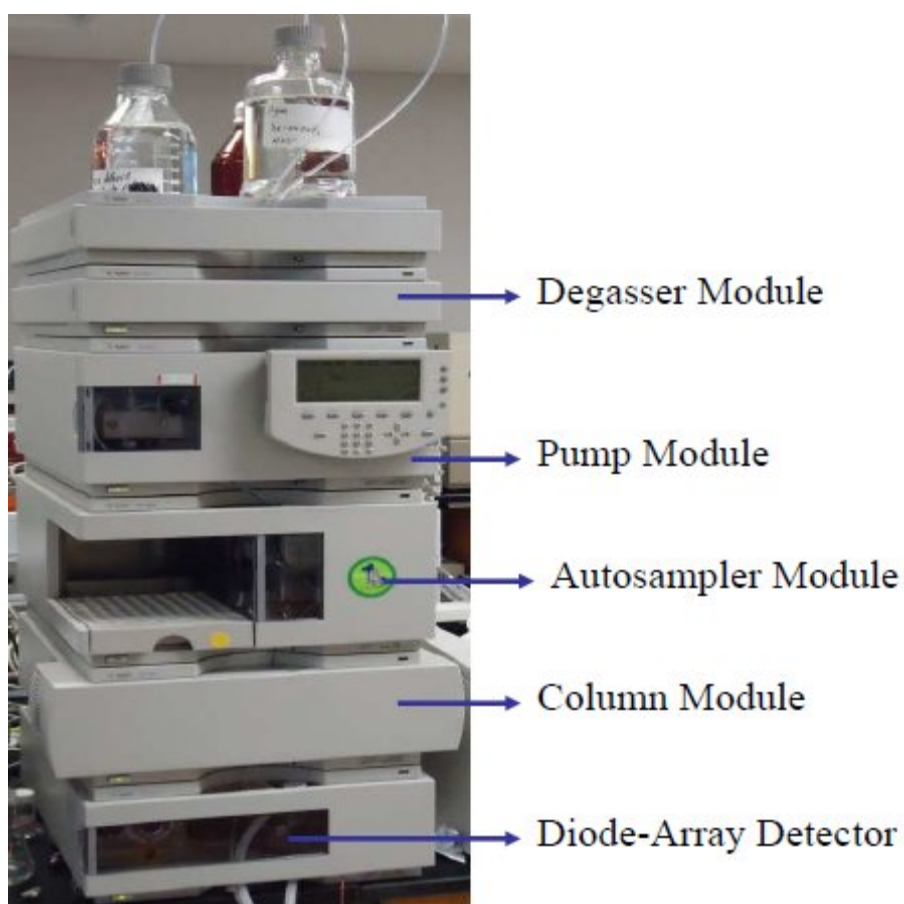
Analytical Balance from OHAUS (uncertainty of 0.0001 g)	Barnstead NANOpure Diamond™ Deionizer (18.1 mΩ-cm)
Adjustable micropipettes (2-10 mL; 100-1000 µL, from Fisher)	Micropipettes Tips, Fisher Scientific (10- 1020µL)
Calibrate pipettes (1.00-10.00 mL transfer capacity, tolerance of ± 0.0006 – 0.02 mL)	Disposal Syringe (5 mL capacity)
Syringe Filter (13 mm, pore diameter 0.45 mm, PVDF sterile)	Filter Paper Whatman
Corning Stirrer/Hot Plate, model: PC-420	pH Meter OAKTON 510 series
Sonicator Branson, model 3510	Weighting Paper, Fisher Scientific
Amber Screw Caps Bottles	Amber Volumetric Flask
HPLC 2 mL amber vials, Agilent	Snap cap 11 mm polypropylene, Agilent



### 3.2 Analytical Instrumentation:

#### 3.2.1 Liquid Chromatography, HPLC

We used a HPLC system series 1100 with an UV-Vis Diode Array Detector. Our HPLC system is divided into five modules as shown in figure 10. A reversed phase with isocratic conditions and a Zorbax Eclipse XDB-C8 column were chosen for our research, in table 5 is a list of parameters.



*Figure 10: HPLC series 1100 from Agilent.*

**Table 7: List of Parameters**

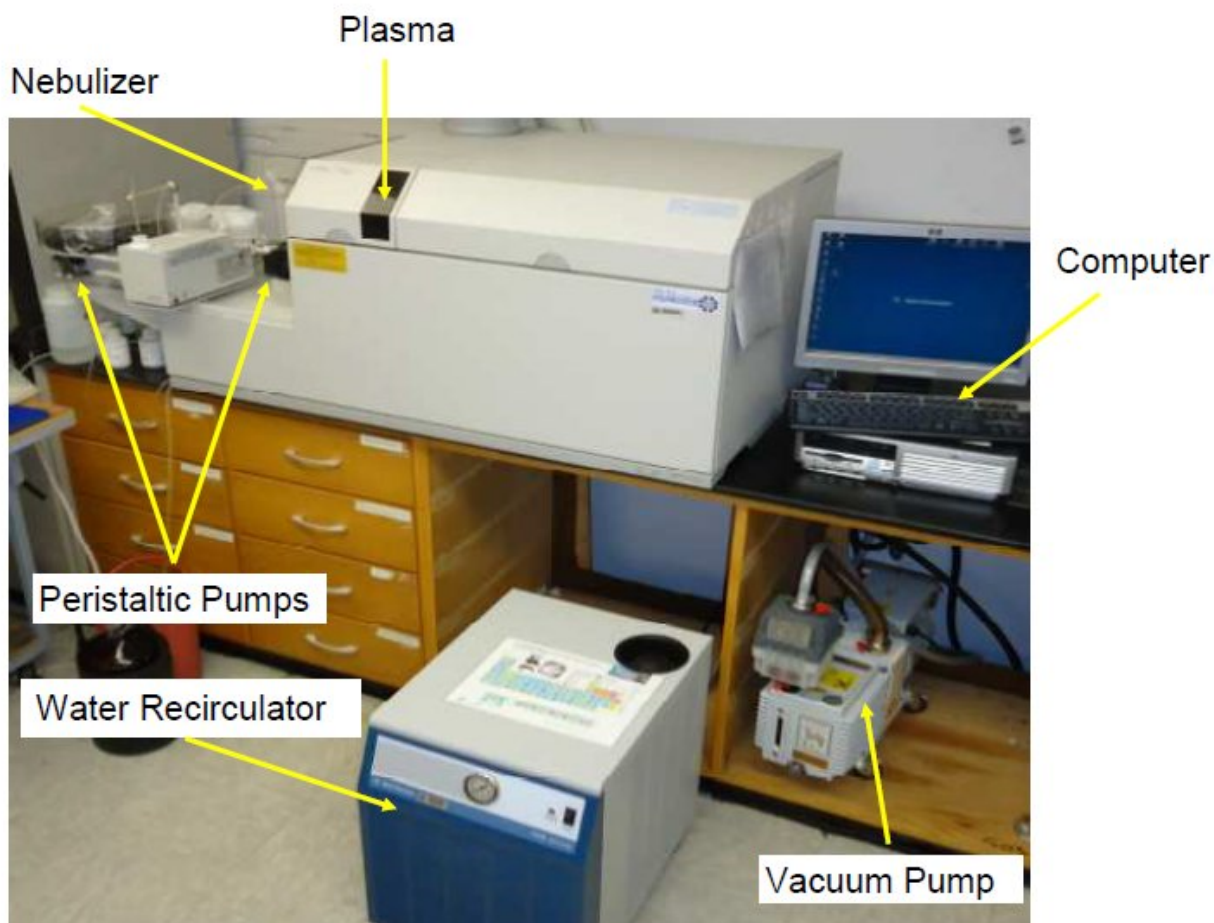
HPLC Parameters	
Stationary Phase	Zorbax Eclipse XDB-C8 column with a particle size of 5µm and 4.6 x 150 mm of internal diameter
Mobile Phase	Isocratic: Acetonitrile and Deionized Water (with 3% Acetic Acid) 50:50
Wavelength (nm)	353
Temperature (°C)	25.0
Flow Rate (ml/min)	1.000
Injection (µL)	50.0

**Table 8: Modules Features**

HPLC Modules	
Degasser Module	Online vacuum degasser offers reduced operating costs and ensures highest instrument performance, with no need for helium.
Pump Module	All pumps come with purge valve, connecting tubing, solvent cabinet, and solvent bottles for convenient startup. <ul style="list-style-type: none"><li>• Isocratic for routine quality control analysis</li><li>• High pressure binary gradient for lowest dispersion and highest precision at low flow rates.</li><li>• Low pressure quaternary gradient for highest flexibility.</li></ul>
Auto-sampler Module	<ul style="list-style-type: none"><li>• Advanced design for fast access to different vial sizes</li><li>• Precise, programmable injection volumes - from 0.1 µL to 1.5 mL</li><li>• Built-in injector program for automated pre-column derivatization.</li></ul>
Column Module	<ul style="list-style-type: none"><li>• Accommodates multiple columns</li><li>• With Peltier heating/cooling for convenient, stable operation above, below and at ambient temperature</li><li>• With column identification module for automated GLP documentation and traceability of column parameters</li></ul>
Diode Array Detector	Two lamps in the HP 1100 Series diode array detector ensure the highest light output from 190 to 950 nm, for the lowest detection limits over the entire wavelength range. The use of 1,024 diodes and an optical slit programmable from 1 to 16 nm ensures the best possible spectral bandwidth.

### 3.2.2 Inductive Couple Plasma – Mass Spectrometry

ICP-MS was used to perform the crumb rubber chemical stability test.



**Figure 11: Diagram of ICP-MS and its components**

### 3.3 Crumb Rubber Chemical Stability Test

Release of metal ions from crumb rubber will hinder its applicability as a clean sorbent, as it can be a source of contamination. In order to evaluate this possibility, 5 g/L aqueous solutions of crumb rubber at pH 1.5, 3.0 and 7.0 were stirred for 48 hours. The pH of these solutions was adjusted adding HNO<sub>3</sub> 10% or NaOH 10%. Solution samples were withdrawn after 48 hours of contact time, acidified with 2.0% of HNO<sub>3</sub> and submitted for copper, cadmium, arsenic, zinc, lead and chromium analyses by ICP-MS. The analytical conditions and standard

procedures were realized following the EPA Method 200.8: Determination of Trace Elements in Waters and Waste Waters by Inductively Coupled Plasma – Mass Spectrometry, rev 5.4. All analyzes were done in triplicate.

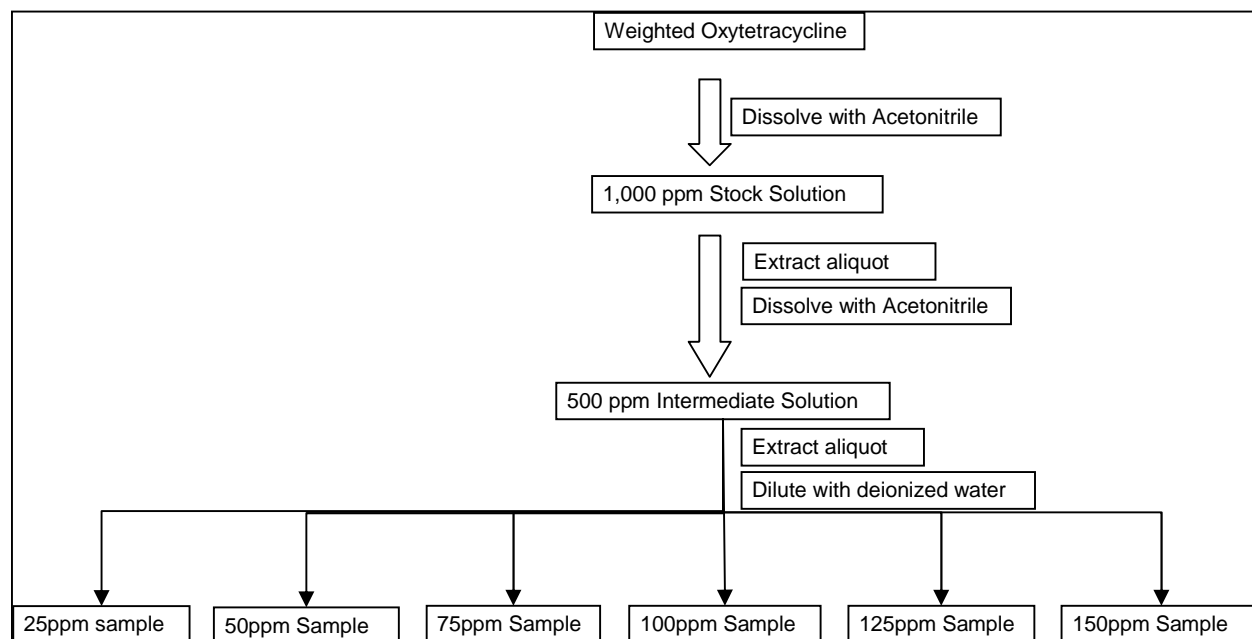
### **3.4 Photolysis Test**

Photolysis or photodecomposition is a chemical reaction in which a compound is broken down by photons. It is defined as the interaction of one or more photons with one target molecule. According to Jiao Shaojun (2008), oxytetracycline is susceptible to photolysis. In order to see the rate of photodecomposition of OTC, we decided to make a photodegradation experiment in order to measure susceptibility to photo-degradation. First samples of 25 and 100 ppm were prepared and placed in Petri-dishes. After that samples were placed in an aluminum foil covered container and then irradiated with an UV-lamp at 365 nm for five hours. Samples were taken each hour and analyzed by HPLC-DAD. With this simple experiment we studied the effect of photo-degradation on OTC.

### **3.5 Sorption Experiments:**

For these experiments we chose to work with a concentration range from 25 ppm to 125 ppm and keeping the amount of crumb rubber constant at 10 g/L.

Amber volumetric flasks of 100 mL capacity were used to prepare the samples. Those samples were transferred to amber bottles of 125 mL and 1.000 g of crumb rubber (mesh 14-20 and 30) was added to each bottles.



***Figure 12: Sample preparation diagram***

The OTC-crumb rubber mixture was mechanically stirred at 600 rpm for 84 hours (using a Corning Stirrer/Hot Plate, model: PC-420) and aliquots were withdrawn at different times and analyzed by HPLC-DAD. In the carbon black's case we only used 0.3000 grams, as this represents the 30% of this component present in crumb rubber. Also the withdrawn aliquots were passed through a 0.45 mm filter, prior to being analyzed by HPLC.

Quality Control (QC) samples were run in all experiments every 10-12 samples and the results were accepted if the error was below 15%. If the error of the QC samples was outside the accepted values a new calibration curve was conducted.

### **3.6 Desorption Tests**

Desorption tests were carried out in order to determine the affinity of OTC for the crumb rubber. After the sorption experiment, the crumb rubber was filtered and placed in contact with solutions containing 1% and 2% of nitric acid, stirred at 600 rpm for 48 hours and analyzed by HPLC-DAD.

## 4. RESULTS AND DISCUSSIONS

### 4.1 Crumb Rubber Chemical Stability Test

Table 9 shows the terminal concentration of metals in solution when 5 g/L of crumb rubber were contacted with deionized water at pH 1.5, 3.0 and 7.0 for a period of 48 hours, along with the maximum levels established by EPA for drinking water.

**Table 9: Released metals from crumb rubber**

Crumb Rubber Stability Test				
	Concentrations in mg/L			
Metals	pH: 1.5	pH: 3.0	pH: 7.0	EPA regulation
Cr	$0.056 \pm 0.002$	$0.031 \pm 0.004$	$0.0024 \pm 0.0020$	0.1
Cu	$0.552 \pm 0.005$	$0.243 \pm 0.006$	ND	1.3
Zn	$11.71 \pm 0.00220$	$5.06 \pm 0.0031$	$1.96 \pm 0.02$	5.0
As	$0.0065 \pm 0.0021$	$0.015 \pm 0.002$	ND	0.05
Cd	$0.0032 \pm 0.0011$	ND	ND	0.005
Pb	ND	ND	ND	0.0

*ND: not detected*

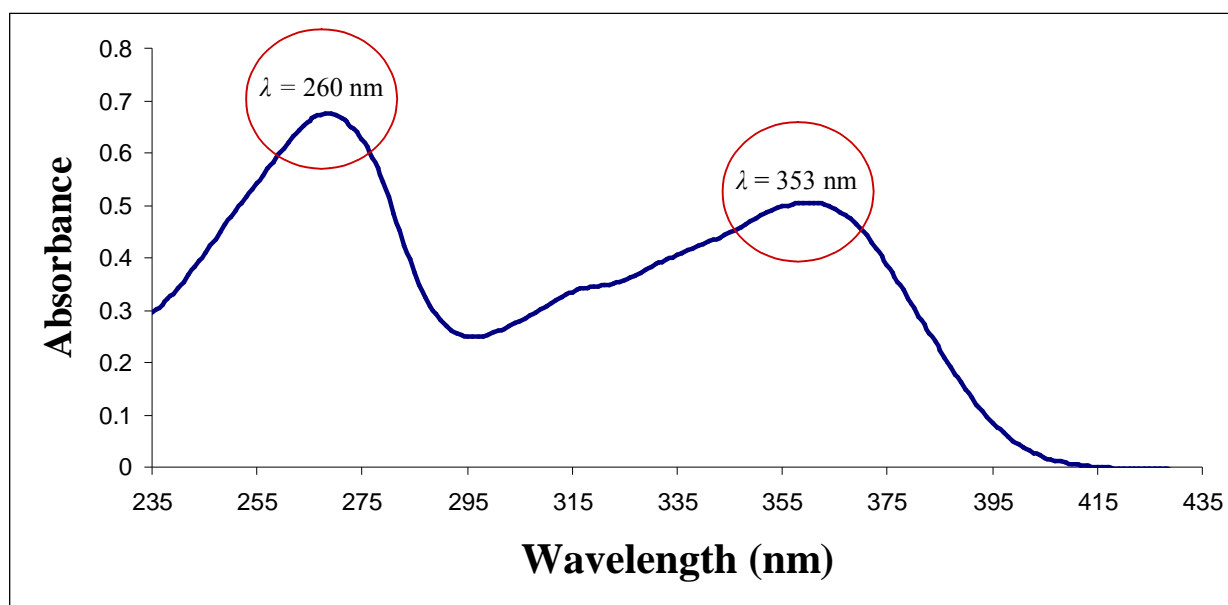
These values shown in table 9 demonstrate the release of metals at various pH. At pH 7.0 the final concentrations were below EPA regulations for drinking water. The zinc (Zn) detected in the sample corresponds to zinc oxide (ZnO), which is a rubber constituent. The more toxic metals such as lead (Pb), cadmium (Cd) and arsenic (As) were not detected.

Only at pH 1.5 and 3.0 we were observed a much larger release of metals, specially zinc. In this work the removal of OTC took place at a relatively long contact time (over 72 hours). Is for this reason the removal of Oxytetracycline took place at pH 7.0; therefore there is little concern about the potential release of metal ions during the sorption process. Another important factor is that the pH of water is approximately 7.0, which also allows us to study the OTC behavior in surface and underground water. Although our research is focused on working with a

pH of 7, this does not mean we should rule out the use of low pH. As it can be seen in the table 7 (above), we can remove a significant amount of Zn, which in turn can be reused in other ways as food additive, concrete industry, etc.

#### 4.2 UV-Vis Measurement

A simple UV-Vis sweep was done in order to determine the maximum absorption wavelength. These results were obtained with a Shimadzu UV-1800 spectrophotometer. The UV-Vis spectrum corresponds to a 25 ppm OTC solution. Two absorption peaks were observed at 260 nm and 353 nm, as shown in figure 13. These absorption peaks are in accordance with the literature.



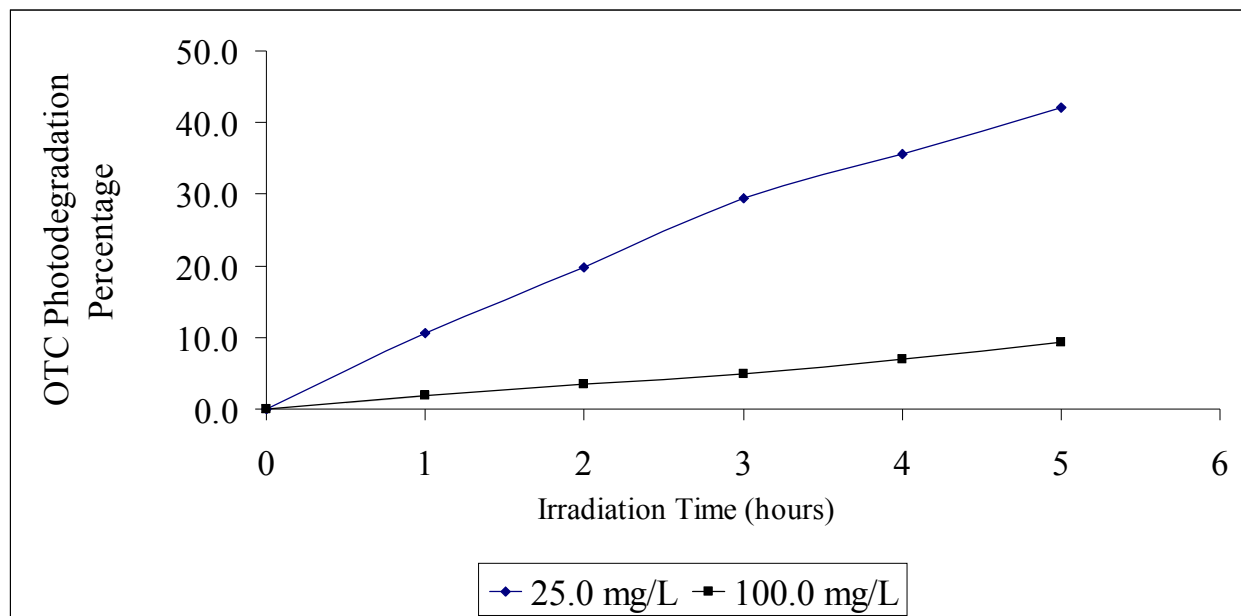
*Figure 13: UV-Vis spectrum for OTC*

The wavelength chosen for this research was 353 nm and we had a better signal and lower noise than when we used 260 nm.



### 4.3 Photolysis Test

Figure 14 demonstrates the rate at which OTC decomposes when irradiated with an UV- lamp at 365 nm and its dependence on the Oxytetracycline concentration



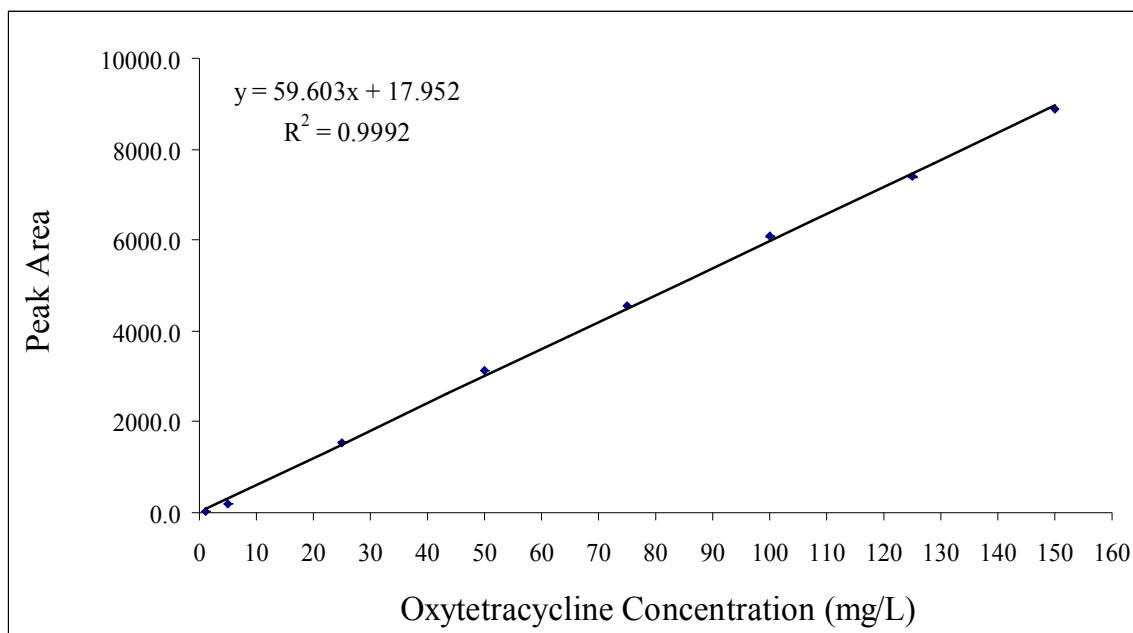
**Figure 14: Oxytetracycline Photodegradation**

As we can see Oxytetracycline is susceptible to light degradation and this effect is inversely proportional to Oxytetracycline concentration. The test shows a photodegradation of 42% and 9% for 25 ppm and 100 ppm respectively, after 5 hours of irradiation. As we can see in the graph shown in figure 14, the OTC photodecomposition rate is higher at lower concentrations, which becomes troublesome when working at lower concentrations.

With this information we decided that it would be best to work with as little light as possible and use amber bottles, as they block 95% of light. This way we can ensure a maximum adsorption of OTC onto crumb rubber and minimize the photo-degradation. We also took into account these photo-degradation results to define our samples concentration range.

#### 4.4 Sorption Experiments

For these experiments we initially used crumb rubber mesh 14-20 and a blank was run, as means to monitor Oxytetracycline photodegradation. Figures 16, 17 allow us to visualize the sorption behavior of Oxytetracycline onto crumb rubber (mesh 14-20).



**Figure 15: Oxytetracycline Calibration Curve (mesh 14-20)**

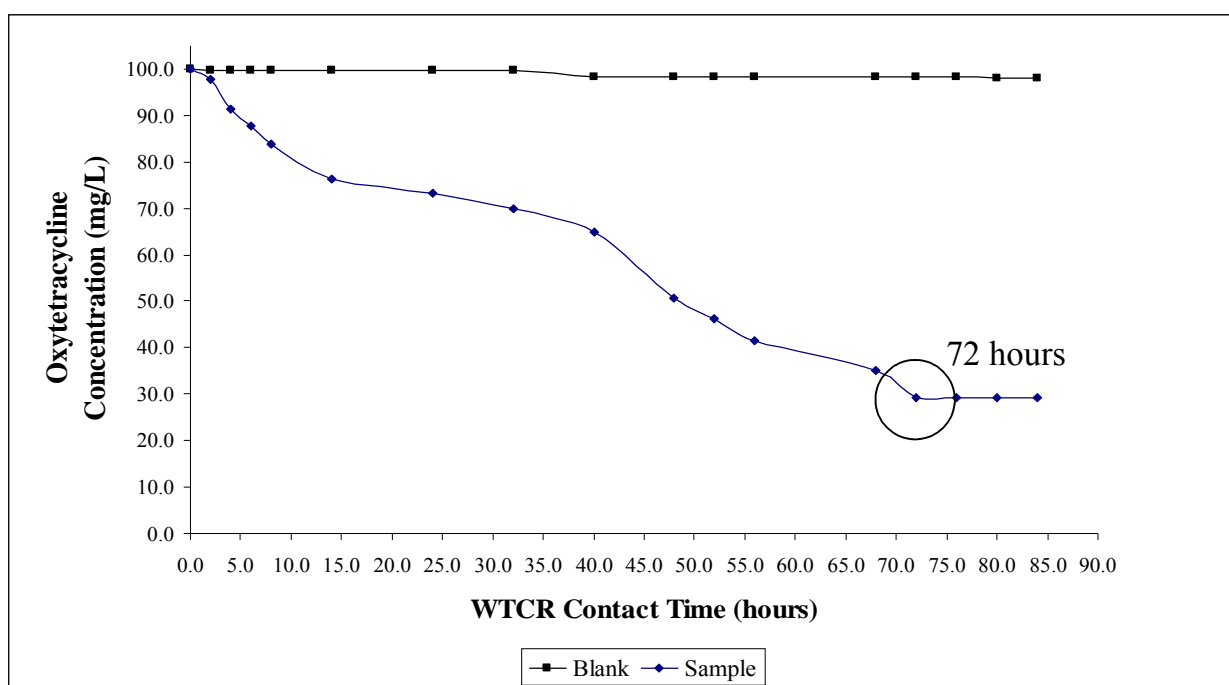
**Table 10: OTC limits of detection and quantification when using WTCR mesh 14-20.**

<i>LOD</i>	<i>0.033 mg/L</i>
<i>LOQ</i>	<i>0.100 mg/L</i>

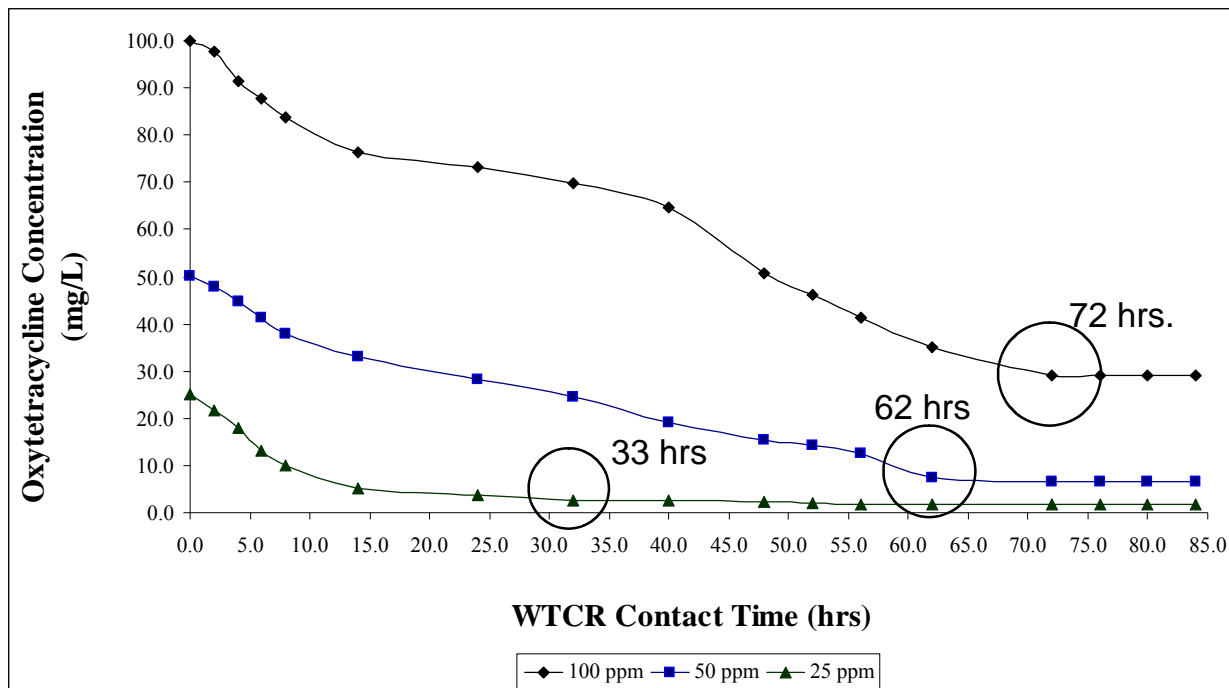
In order to determine our LOD and LOQ for the method a calibration curve was constructed. As we can see in figure 16 our calibration curve had an  $r^2 = 0.9992$ , which tell us that there is a good linear correlation between the OTC concentration and the Peak Area. Using the calibration curve we proceeded to determine our limits LOD and LOQ shown in table 10

using the International Conference on Harmonization (ICH) method. These values were within our expectations.

In the adsorption experiments using WTCR mesh 14-20 shown in figure 17 we can observe several tendencies: (1) by working with amber bottles we reduced OTC photodecomposition and, (2) a significant amount of OTC,  $\sim 70$  mg/L was removed from solutions, (3) the process as slow, and (4) the equilibrium was reach after 72 hours.

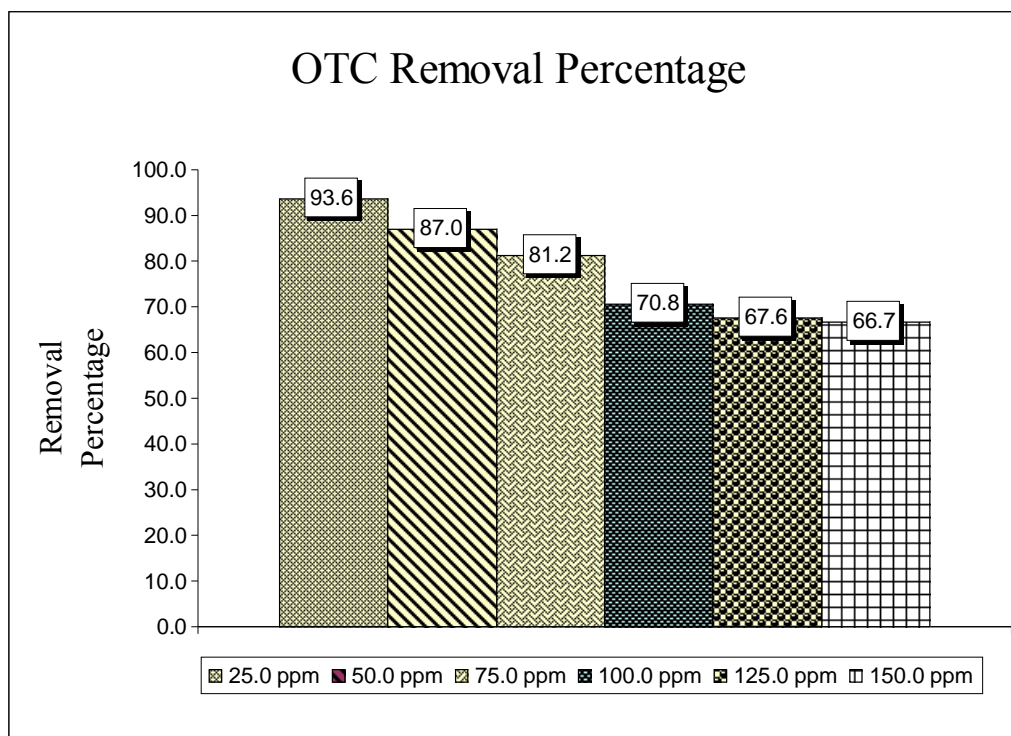


**Figure 16: Adsorption Experiment for 10 g/L of WTCR (mesh 14-20)**



**Figure 17: Effect of OTC initial concentration using 10 g/L of WTCR**

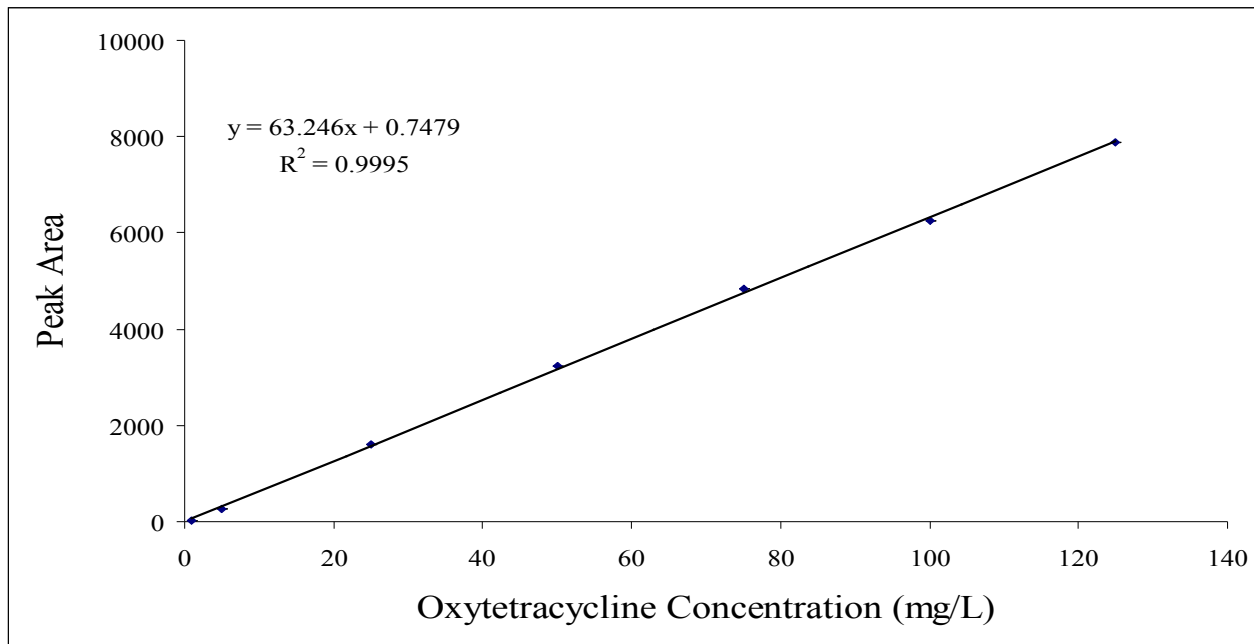
Figure 17 shows the effect of OTC concentration in the adsorption experiment when using 10g/L of WTCR and the starting concentrations are of OTC are 25, 50 and 100 ppm. It is evident from this data that the lower the concentration of OTC, the faster the equilibrium time is reached. At lower OTC starting concentrations, not only does the equilibrium time is reached faster, but also the percentage removal is increased as the initial concentrations are decreased from 100 to 25 ppm as shown in figure 18.



**Figure 18: Relation between OTC concentration and 10 g/L of WTCR**

With the results shown in figures 17 and 18 we can conclude that WTCR has good sorption affinity for OTC, with removal percentage up to ~ 94 % depending in the initial concentration. These results suggest that WTCR has the potential to be used as an alternative method for the treatment of wastewaters contaminated with OTC.

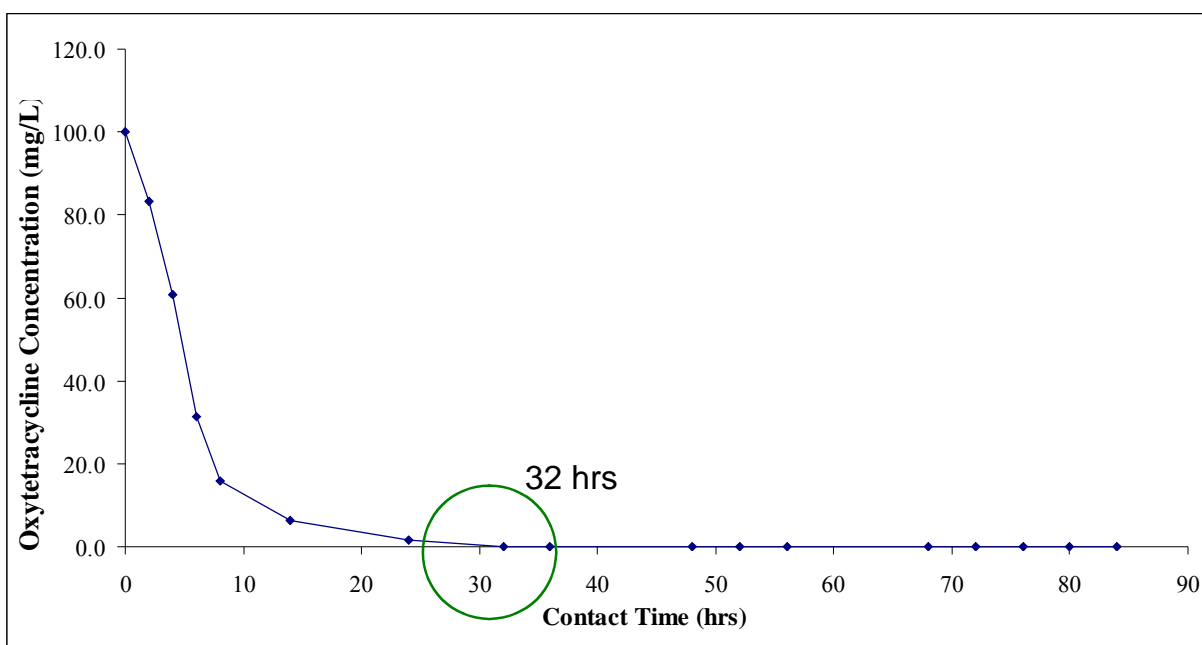
The participation of Carbon Black (CB) present in WTCR in the sorption process of OTC in aqueous solution is suspected. According to previous works carbon black particles exhibit a quite large area and are well known for it sorption capability. CB is produced by the incomplete combustion of heavy petroleum products and has a high surface-area-to-volume ratio. With this in our minds we decided to design an adsorption experiment to evaluate the role of carbon black in the sorption process of OTC in the WTCR matrix. Instead of using crumb rubber, 0.3000g of carbon black was used since this is approximately the concentration found in the WTCR matrix.



**Figure 19: Oxytetracycline Calibration Curve (carbon black)**

**Table 11: OTC limits of detection and quantification when using carbon black.**

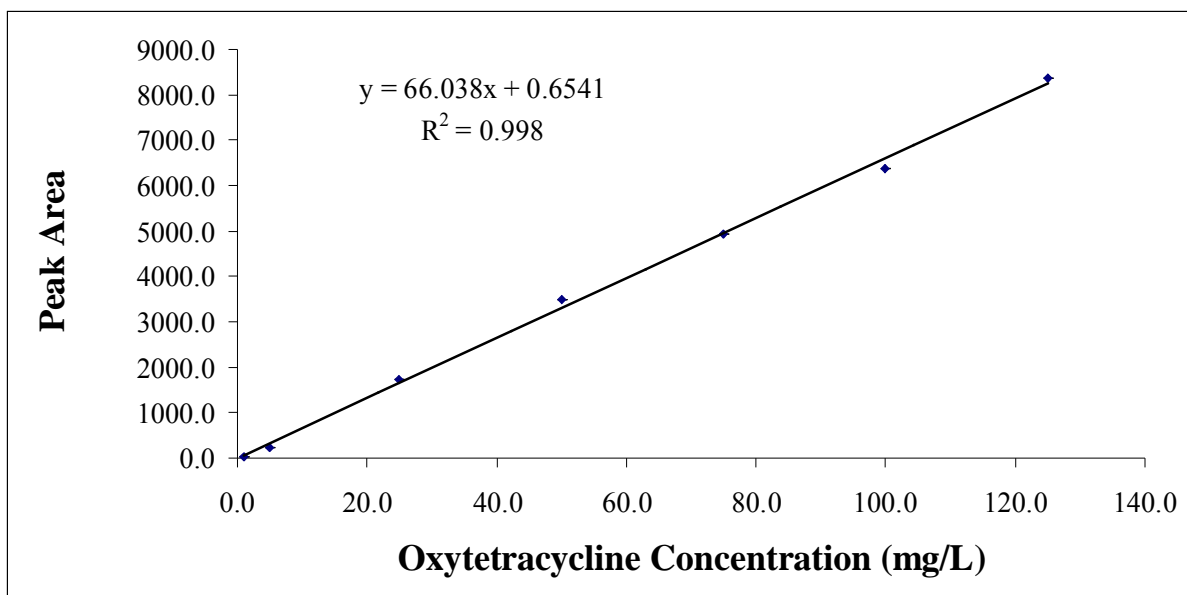
<i>LOD</i>	<i>0.034 mg/L</i>
<i>LOQ</i>	<i>0.098 mg/L</i>



**Figure 20: Adsorption Experiment when using 3 g/L of Carbon Black**

The same procedure used in the WTCR mesh 14-20 was evaluated for the sorption capacity of carbon black in aqueous solutions. A calibration curve was constructed and an  $r^2 = 0.9995$  with limits of detection and quantifications were similar to those previously obtained were achieved using the same ICH method. As illustrated in figure 20, carbon black has a very good adsorption capability for OTC. Carbon black adsorbed nearly 100% of the OTC in our sample in a fraction of the time (30 hours). The results obtained from the carbon black experiment suggest that carbon black plays an important role in the mechanism of OTC sorption onto WTCR.

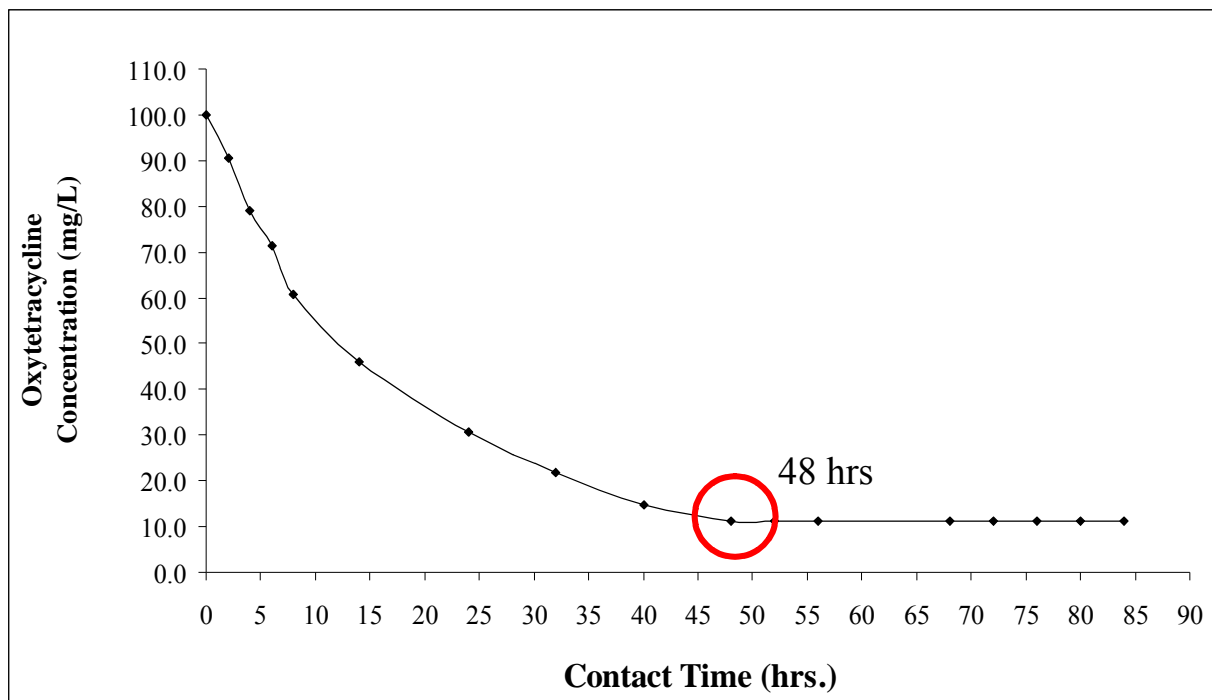
The previous experiments using WTCR 14-20 (figures 16 and 17) showed a slow process for the removal of OTC when compared with CB under the same conditions. These results suggest that probably the slow removal rate when compared with WTCR can possibly be attributed to the difficulty reaching the carbon black sites within the WTCR matrix. To test our hypothesis, a new set of experiments were developed using WTCR mesh 30, which has significantly smaller grains with an increased surface area. Increasing the exposure of the carbon black particles within the WTCR matrix is expected to significantly increase the removal of OTC molecules and at the sometime increase the sorption into the aqueous solution.



**Figure 21: Oxytetracycline Calibration Curve (WTCR mesh 30)**

**Table 12: OTC limits of detection and quantification when using WTCR mesh 30.**

<i>LOD</i>	<i>0.034 mg/L</i>
<i>LOQ</i>	<i>0.098 mg/L</i>

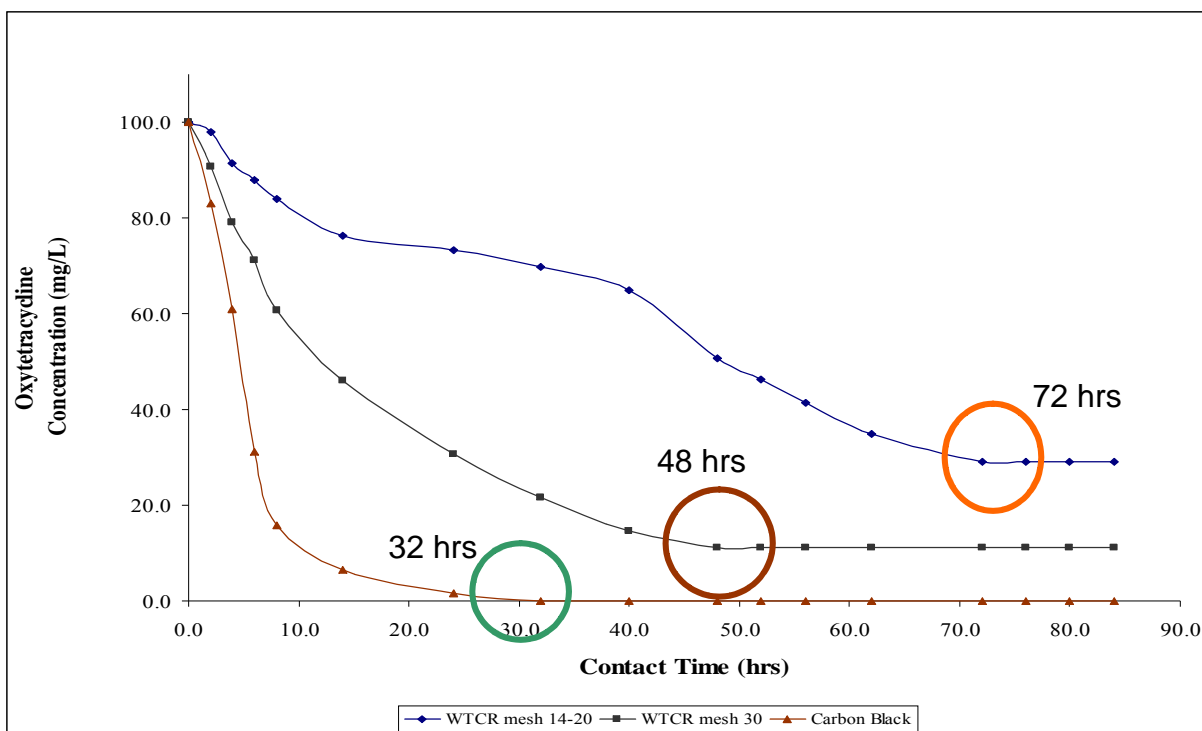


**Figure 22: Adsorption Experiment using 10 g/L (WTCR mesh 30)**



Just like in the previous experiments a calibration curve was constructed with an  $r^2 = 0.998$  and LODs and LOQs were similar to the previous one. As demonstrated in figure 23 by reducing the mesh size we can potentially increase WTCR adsorption of OTC. This is possible due to a larger surface area with exposed carbon black in WTCR mesh 30 than when compared with mesh 14-20.

The removal of Oxytetracycline at pH 7.0 was highly efficient for mesh 14-20 and mesh 30. As we can clearly see mesh 30 had a better sorption and faster equilibrium times than mesh 14-20. It was also noted that crumb rubber worked best at low concentrations as previously discussed and shown in figure 18.



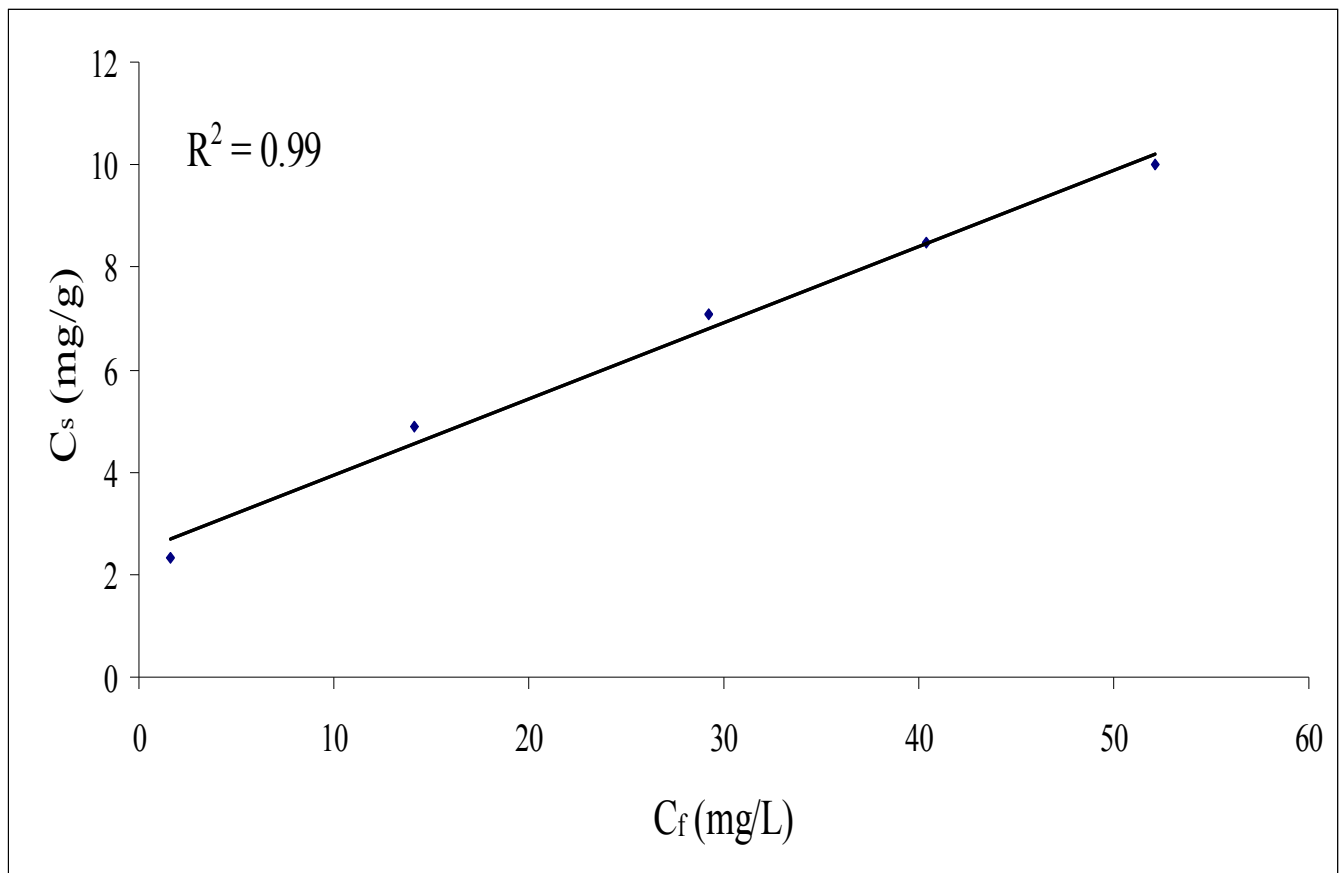
**Figure 23: Comparison between mesh 14-20, mesh 30 and carbon black**

Figure 23 shows a correlation between WTCR at mesh 14-20 and 30 and CB. From this figure 21 it can be determined that CB is more efficient than crumb rubber for the removal of

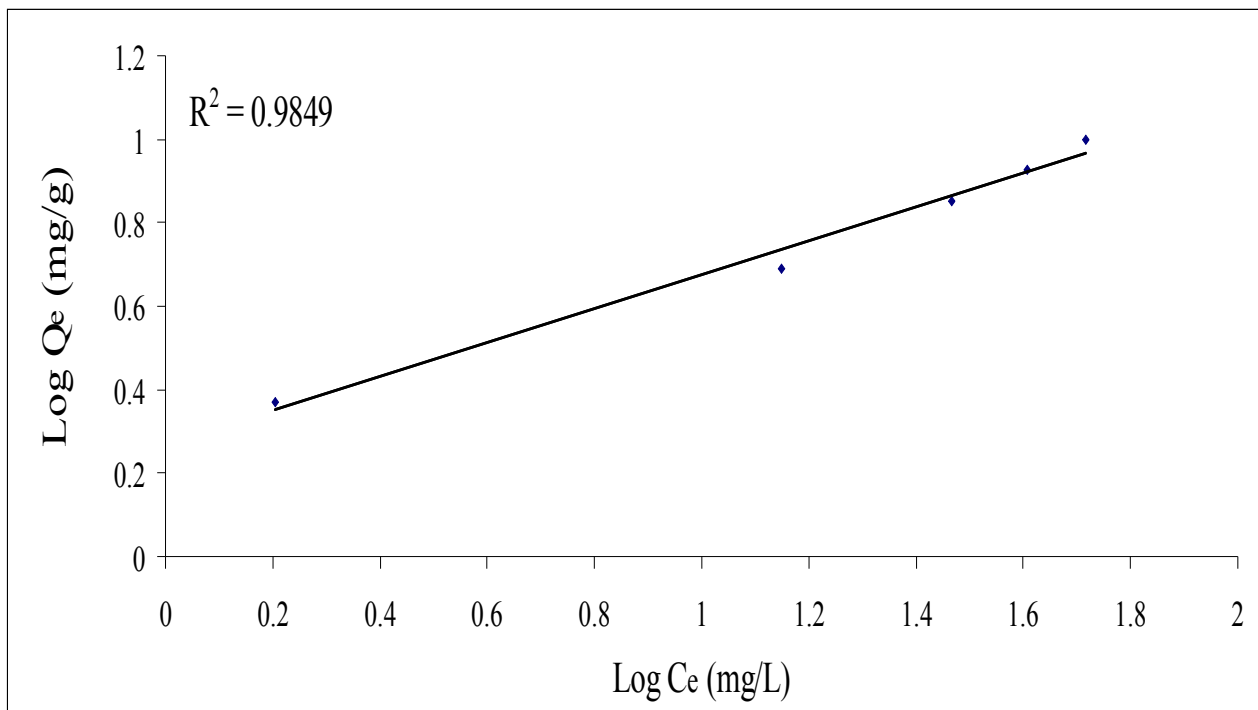
OTC at various concentrations. The problem with CB is that is very difficult to work with since it will stain everything that comes in contact with it. Also CB is more expensive than WTCR, thus make it a cost-effective alternative.

#### 4.5 Sorption Isotherms

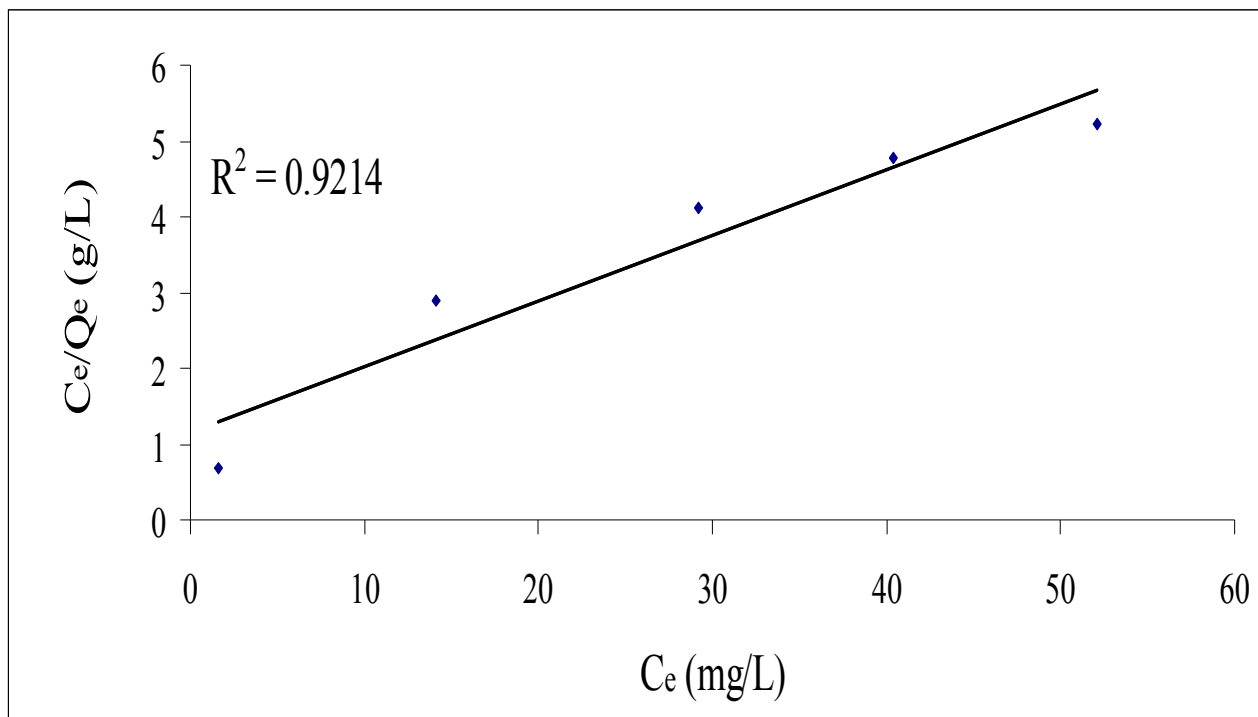
Three different isotherms were applied to our research results and are shown in figures 23, 24 and 25.



**Figure 24: Linear Isotherm**



**Figure 25: Freundlich Isotherm**



**Figure 26: Langmuir Isotherm**

The Linear Isotherm is a well fitted model, as it shows a great linear coefficient correlation ( $r^2 = 0.99$ ). The Freundlich Isotherm fits better the sorption behavior than Langmuir Isotherm, as it has the highest  $r^2$  (0.985) and goes in accordance with the theory. Table 13 shows a complete list of parameters for each isotherm.

**Table 13: Linear, Freundlich and Langmuir Parameters**

Linear		Freundlich			Langmuir		
$K_d$ (g/L)	$r^2$	$n$ (g/L)	$K_f$ (mg/g)	$r^2$	$Q_0$ (mg/g)	$B$ (L/mg)	$r^2$
$0.1486 \pm 0.002$	0.99	$2.52 \pm 0.187$	$1.89 \pm 0.0810$	0.985	$11.1 \pm 0.0810$	$0.091 \pm 0.0021$	0.921

$K_d$ : distribution equilibrium coefficient  
 $n$ : Freundlich parameter  
 $K_f$ : Freundlich constant; loading capacity

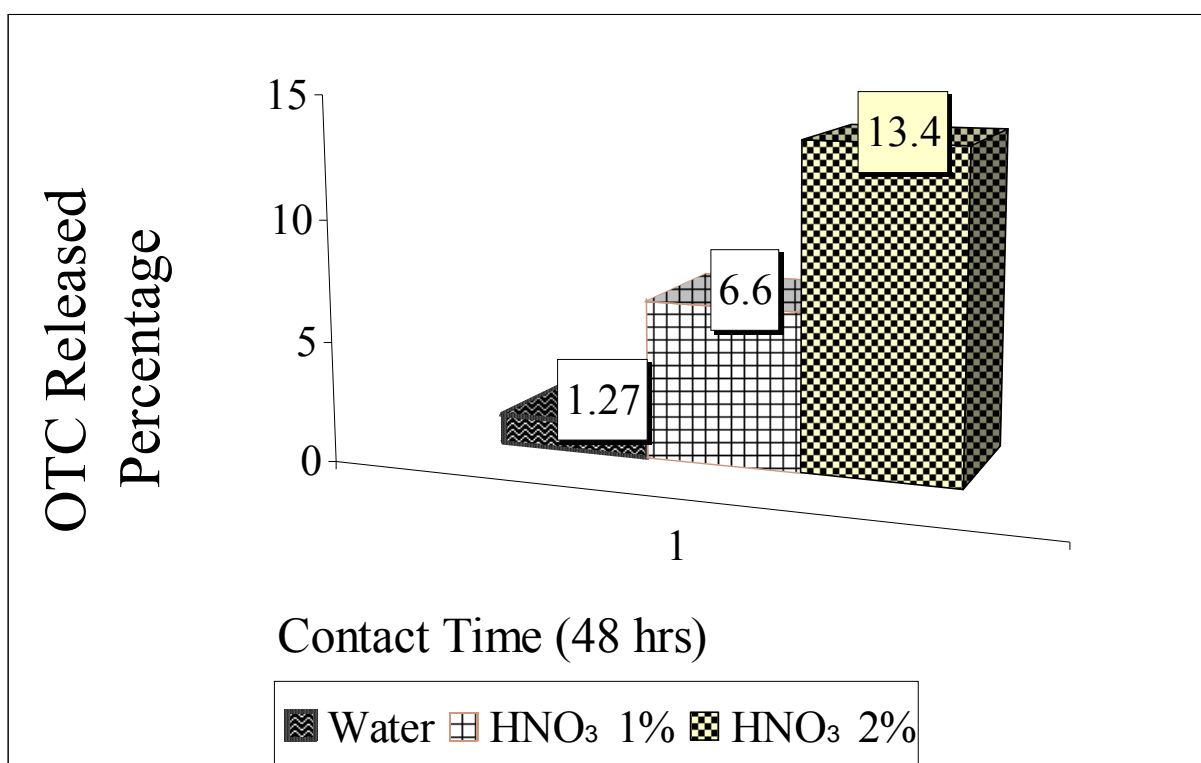
$Q_0$ : maximum amount sorbed  
 $B$ : Langmuir constant  
 $r^2$ : R square

For the Linear Isotherm  $K_d$  represents the distribution equilibrium coefficient. This value allows us to see how fast the equilibrium in the system is reached. In our case we obtained a  $K_d = 0.1486 \pm 0.002$ . We considered this value be lower than expected when compared with related works using crumb rubber as a adsorbent for organic compounds like Alamo-Nole (2006), which reported a  $K_d = 1.3 \times 10^3$  for Xylene.

In the case of the Freundlich Isotherm  $n$  parameter which is higher than 1 indicates that crumb rubber works better at lower concentrations of Oxytetracycline, as shown in figure 18. This was also observed in Alamo-Nole (2006) work, in which the  $n$  parameter for the Freundlich Isotherm is lower than 1 for all organic compounds analyzed. Another parameter that was evaluated using the Freundlich Isotherm was  $K_f$ , which indicates the loading capacity of the sorbent. Our  $K_f = 1.89 \pm 0.0810$ , which is higher than the  $K_f$  reported by Lama Sameeh Mohammad Awartani in 2010.

#### 4.6 Desorption Test

In order to evaluate the reuse of crumb rubber, a desorption process was tested. The crumb rubber to be subjected to sorption experiments were filtered and treated nitric acid. Aliquots were withdrawn and analyzed at different hours to see the release of OTC. Figure 22 shows the amount of OTC released when in contact with nitric acid 1% and 2% for a period of 48 hours. As demonstrated below the sorption of OTC onto crumb rubber has poor reversibility, which makes the final disposal of the crumb rubber a safe option, discarding any possibility to release the sorbed species back into the environment.



**Figure 27: Oxytetracycline Release from WTCR**

This data suggest that the desorption process was not due to an ion exchange mechanism. In the near future we will be working to see if other solvents (e.g. methanol or acetonitrile) would have better desorption mechanism.

## 5. CONCLUSIONS

- ❖ There was no significant release of toxic metal ions when crumb rubber was placed in contact with deionized water at pH 7.0 and stirred for 48 hours at 600 rpm.
- ❖ At pH 1.5 and 3.0 the amount of metal ions released increased greatly, especially zinc which exceeded EPA regulations.
- ❖ Oxytetracycline photodecomposition rate increases at lower concentrations, thus making difficult working at lower concentrations.
- ❖ WTCR has excellent capability as sorbent for antimicrobial compounds.
- ❖ The increased sorption capability of WTCR mesh 30 over mesh 14-20 was probably due to having a larger surface area with exposed carbon black. However mesh 14-20 is preferred when working with continuous systems, as it can be more easily packed into columns and/or filters.
- ❖ The sorption process of Oxytetracycline onto crumb rubber has poor reversibility, thus making the disposal of WTCR a safer option or any other alternative such as tire-derived fuel or rubberized asphalt.
- ❖ The sorption process is by favored the Freundlich model over the Langmuir model.
- ❖ The desorption process of OTC from WTCR was not due to an ion exchange mechanism

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