World Journal of Pharmaceutical research

Volume 3, Issue 2, 1568-1578. Research Article ISSN 2277 – 7105

DETERMINATION OF OXYTETRACYCLINE RESIDUES IN UNTREATED AND TREATED DRINKING WATER IN BINDURA TOWN BY RP-HPLC-UV VISIBLE SPECTROMETRY AFTER ULTRASONIC ASSISTED DISPERSIVE SOLID PHASE EXTRACTION (UA-DSPE)

[1,2] Pamhidzai Dzomba*, [2]Mark F. Zaranyika, [2]Jameson Kugara, [1]Tonderai Zhanda

[1]Chemistry Department, Bindura University of Science Education, P. Bag 1020, Bindura, Zimbabwe

[2]Chemistry Department, University of Zimbabwe, P. O. Box MP167, Mount pleasant, Harare, Zimbabwe.

Article Received on
19 November 2013
Revised on 15 December 2013,
Accepted on 12 January 2014

***Correspondence for Author:**
Dr. Dzomba Pamhidzai
Bindura University of Science Education, Chemistry Department, P bag 1020 Bindura , Zimbabwe.

ABSTRACT

The study was concerned with determining levels of oxytetracycline in untreated and treated drinking water in Bindura town. Ultrasonic Assisted Dispersive Solid Phase Extraction (UA-DSPE) was employed to extract oxytetracycline from its matrix. Quantitative analysis was carried out using HPLC coupled to a UV-Vis detector set at 360nm. The overall mean recovery from water samples was 98.6 ± 3.66%. The Limit of Detection (LOD) and Limit of Quantification (LOQ) were found to be 11.55 and 35.05 ng/ml respectively. Oxytetracycline was present in all the untreated water samples from Bindura with values ranging from 0.0611 ± 0.000865µg/ml to 0.1495±0.001085µg/ml. No oxytetracycline was detected in most treated water samples except for two samples from Chipadze and Chiwaridzo. Chipadze recorded 0.0711 ± 0.000071µg/ml whilst Chiwaridzo had 0.0660 ± 0.000798µg/ml and these values are even higher than some obtained for untreated water. This anomaly may be due to contamination of treated water with sewage as these areas are frequently faced with serious water and sewer pipe bursts. Unregulated chicken raring is also very high. It may be possible that oxytetracycline which is the widely used antibiotic may have been washed from the vegetable gardens by runoff. Occurrence of oxytetracycline in both treated and untreated

water may pose a health risk to the general public. Microbial resistance may develop and reduce successes of pharmaceuticals that share the same structure activity relationship with oxytetracycline.

Keywords: Oxytetracycline, Ultrasonic Assisted Dispersive Solid Phase Extraction, HPLC-UV, microbial resistance.

INTRODUCTION

Recent increase in demand for food as a result of the increase in the world population has resulted in expansion of unregulated poultry and livestock farming particularly in Africa (Sanginga et al., 2003; Franke et al., 2010). Large amounts of tetracycline antibacterials are administered to animals in order to treat infectious diseases. They are also used as growth promoters and prophylactics. Oxytetracycline is a natural or synthetic antibiotic of great antiquity used to correct breathing disorders in livestock, treat respiratory infections of sinuses, wound infections and pneumonia. It causes an increase in biomass when used in the milligram per kilogram range therefore it is also used as agri-feed additive. Oxytetracycline is the most commonly used antibacterial in Zimbabwe. It is sold under trade names terranox or teramycin. Pharmaceuticals administered to both humans and animals are becoming a major concern as far as environmental issues are concerned. Recent researches including those done by (Wen et al., 2009; Jia et al., 2009; Yang et al., 2011; Shafrir and Avisar 2012) shows that antibiotics are showing up in soil, groundwater, as well as drinking water. This poses a threat to human and animal health. Both humans and animals are not able to fully metabolize drugs administered as medicine hence a greater portion is excreted unchanged. These compounds eventually find their way to the aquatic environment. Even though current detected levels are very low to raise alarm however their continued presence in the environment has been shown to trigger microbial resistance (Yang et al., 2011). Resistance developed in the environment in non pathogenic organisms can be transferred to pathogenic organisms in humans through drinking water and the food chain. There is also a general fear that persistence of antibiotics in aquatic environments could potentially reach levels that are toxic to humans and other organisms. Other chronic effects on humans include nephrotoxicity, hepatotoxicity, skin hyperpigmentation in areas exposed to the sun and hypersensitivity reactions (Navratilova et al., 2009). Tetracyclines antibacterials have also been implicated in the development of hypouricemia, hypokalemia, proximal and distal renal tubular acidosis. If they are used by

children of 0-8 years or by pregnant women there is a risk of developing secondary tooth discoloration (Navratilova et al., 2009).

Oxytetracycline is an emerging aquatic micro-pollutant and is attracting renewed attention (Sarmah et al., 2006; Wen et al., 2009). Recent reports show that antibacterial agents have an impact on aquatic life; underground and surface waters (Wang and Yates, 2008; Jodeh and Awartani 2011). Halling-Sorensen, (1998) reported that oxytetracycline inhibited nitrification. Several researches have shown that antibiotic-resistant genes can be built up and transferred among microorganisms in the environment (Kummerer 2009; Diwan et al 2010; Sivri et al 2012) by adapting resistance-encoding genes. Researches in the occurrence, fate and implications of antibacterials in the aquatic environment are just beginning. Thus current sewage, industrial and water treatment systems are not designed to look for such pharmaceutical agents and do not necessarily remove them (Batt et al 2006; Gomez et al., 2007). Currently water treatment technologies produce water that satisfies current regulatory standards. Oxytetracycline is not among the list of regulatory pollutants in drinking water. Low to high levels are legally discharged into surface water. Even though researches in pharmaceutical pollution of the environment have just begun pharmaceutical agents such as oxytetracycline have been released into the environment for several years. Information on their fate and effect is still not well researched such that there are no safety limits for tetracycline antibiotics in surface and drinking water. Currently much of severance studies to screen for oxytetracycline have been performed in Europe and Asian countries. Very few severance studies have been performed in Africa despite a sharp rise in the use of antibacterial agents. Thus the aim of this study was to assess the occurrence of oxytetracycline antibacterial in treated and untreated water from Bindura town. This study is important as it may lead to changes in polices. Currently most countries do not make it mandatory for those involved in processing and selling drinking water to look for tetracyclines or design treatment processes that can remove them.

MATERIALS AND METHODS

Oxytetracycline hydrochloride (95%) standard and (57738-U-SUPELCO supelclean primary secondary amine (PSA) were purchased from Sigma Aldrich, Germany, Oxalic acid, Nitric acid (5%), Methanol, (Acetonitrile HPLC solvents), Ammonia, Sulphuric acid (80%), Na_2EDTA were of analytical grade obtained from Merck Chemical Company.

Water sampling

All water sampling containers were cleaned by soaking in 5% HNO_3 for 48 hours. The containers were washed with detergent and rinsed using distilled water followed by acetone and dried. These containers were then rinsed three times with the sample water at the site prior to collection. Sampling sites were Astra Campus, Town, Aerodrome, Main Campus, Chipadze, Cottco grounds, New Site and Chiwaridzo Fig 2. Samples were placed in brown bottles and transported in a cooler box straight to the laboratory where they were stored in a refrigerator at around 4°C waiting analysis.

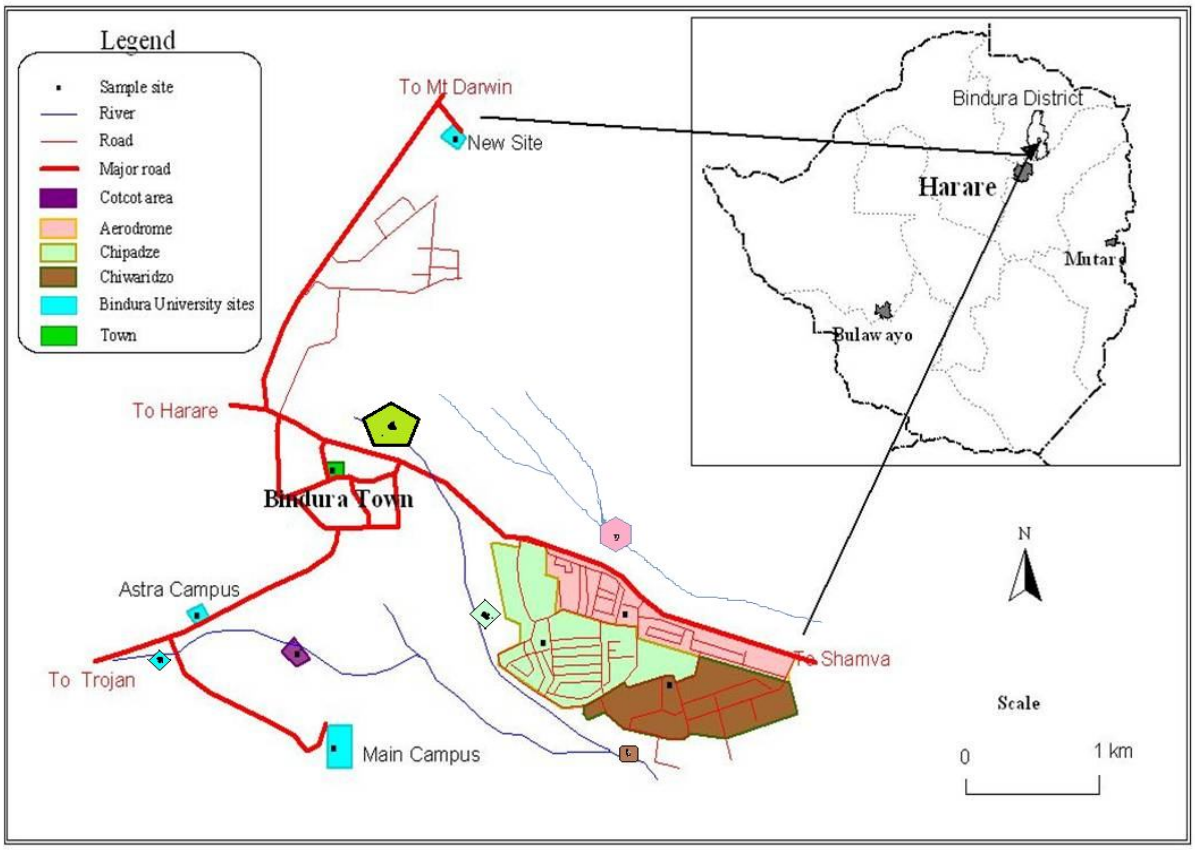

Fig 1. Sampled areas in Bindura town

Sample preparation

Samples were filtered to remove suspensions. Five millilitres of McIlvaine buffer (pH 4) and 5ml of 0.01 EDTA were added to chelate any metals present prior to extraction. Pre-concentration and clean up was done using dispersive solid phase extraction (DSPE) Filtered water samples were vigorously shaken with 10ml of acetonitrile in a separating funnel. Magnesium sulphate and sodium chloride 0.5g each was then added to displace the extraction equilibrium towards the organic phase. The contents were centrifugation at 3000 rpm for 10 minutes and the organic supernatants were transferred to a conical flask followed by addition of 40mg of primary secondary amine sorbent material (57738-U-SUPELCO supelclean PSA)

to remove interferences such as humic acid and proteins. The mixture was ultrasonicated for 15 minutes and centrifuged at 3000 rpm for 10 minutes. The organic supernatants were collected evaporated to almost dryness under vacuum and then redissolved in 500 µl of methanol. The contents were filtered through a 0.45 µm glass Millipore filters to remove any particulate matter and then placed into amber vials and stored in a fridge until HPLC-UV analysis. At weighing stage analytical balance was calibrated before readings were taken, this was achieved through the use of a 200 g standard weight and a reading of 200.00003 g was obtained which fell within the accepted range of 200 ± 0.00005 g. Verification was also done using a 0.1g weight which gave a reading of 0.10002 g which also fell within the range of 0.1 ± 0.00005 g. The pH meter was also calibrated using pH 4 and 7 buffer solutions prior to taking readings.

Standard solution preparations

Stock solutions for oxytetracycline were prepared by weighing exactly 0.1 g of the substance into a 100 ml volumetric flask and methanol was added to the mark making a concentration of 1×10^{-3} g/ml. Working standard solutions were then made from these stock solutions by appropriate dilutions using methanol. The absorbance at maximum wavelength for each compound was determined using a UV-Vis instrument, GENESYS 10S UV-Vis v4.003 2L9Q129001.

HPLC analysis

Analysis for oxytetracycline was performed on a Varian HPLC UV prostar 325 equipped with a Rodyne manual injector with a 20 mL loop and a UV detector, prostar 325. The detector was controlled remotely by the Varian Star/ Galaxie Chromatography Workstation software. All the analytes were separated using HPLC Varian Microsorb MV 1005 packed C18 columns 250´ 4.6 mm id, 5 mm particle size, 100 Å SPELCO. The separation mode used was isocratic. Different mobile phase mixtures were tried basing on information from previous studies. The best mobile phase mixture was prepared by mixing methanol, acetonitrile and 0.01 M aqueous oxalic acid at pH 3.0 (adjusted using concentrated NH_3) in the ratio of 1:1.5:5. A sonicator was used to mix and remove air bubbles. Wavelength of 360 nm was used to analyze samples. Sample injection volume was 10 µL. Flow rate of mobile phase was kept at 1.0 ml/min. Ambient room temperature was used on the column. A typical chromatogram obtained is shown in Fig 1 below.

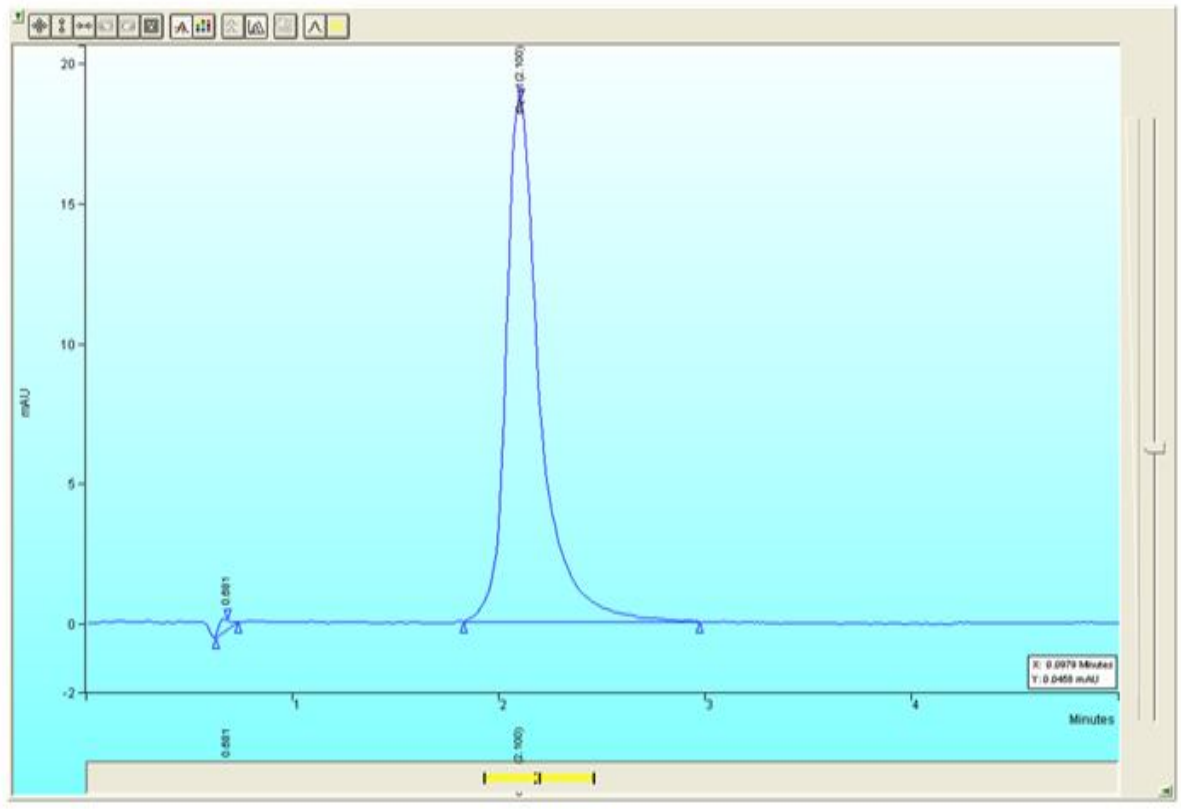

Fig 1. A typical oxytetracycline chromatogram retention time 2.1 minutes

RESULTS

The use of Ultrasonic Assisted Dispersive Solid Phase Extraction (UA-DSPE) helped in improving the detectability of oxytetracycline which could have been difficult at low concentrations and complex matrix (R. Vallejo-Rodriguez et al 2011). DSPE extraction involved shaking the sample with acetonitrile and addition of salts to facilitate phase change. The use of primary secondary amine trapped interferences such as humic acid and proteins which may complex with oxytetracycline and complicate their extraction. The overall mean recovery was 98.6 ± 3.66%. Ultrasonication provided the extra energy required to break the bond between the analyte and the interferent. Most of the samples were analysed five times except samples obtained from new site which was analysed only four times. Sample bottle fell and broke during analysis. Results for validation analysis Table 2 revealed a very strong correlation between the concentration and absorbance with R^2 values of 0.998. The calibration curve for the method generated by plotting analyte peak area versus concentration was linear at the range 0.01-1 µg/ml. The LOD and LOQ which were calculated as three times and 10 times signal to noise ratio were found to be 11.55 and 35.05 ng/ml respectively.

Precision as a parameter for quality was estimated by calculating standard deviation for 3 replicate samples. Computed standard deviations obtained in the present study are all within the accepted range Table 1-2. Specificity was assayed with endogenous interferences by extracting and analyzing blank river water and sediment from ten different sources that were away from sources of pollution. Chromatograms recorded were free of interfering peaks both in the extract and blank. Peak purity assessed by the Varian Star/ Galaxie Chromatography Workstation software revealed that all peaks purity levels were equal to or greater than 99%.

Table 1 Results of validation parameters.

Validation parameter	OTC	
Linear regression equation	y = 30625x + 60528	
R^2	0.998	
Linear dynamic range	0.01 – 1µg/ml	
Limit of detection (ng/ml)	11.55 ng/ml	
Limit of quantification (ng/ml)	35.05 ng/ml	
Level of spiked samples (µg/ml)	0.01	1.00
Recoveries (%)	98.61	98.56
Relative standard deviation (%)	3.78	3.59

Results obtained in the survey showed that oxytetracycline was present in most untreated water sources Table 3. The antibiotic was detected frequently in storm drains and upper reaches of the stream. On moving down downstream no antibiotic was detected. The highest value was 0.1495 ± 0.001085µg/ml and was detected from Aerodrome water samples. The lowest was 0.0611 ± 0.000865µg/ml which was recorded for Main Campus samples. No oxytetracycline was detected in drinking water from six sites except samples from Chipadze and Chiwaridzo Table 4. Chipadze recorded 0.0711 ± 0.000071µg/ml whilst Chiwaridzo had 0.0660 ± 0.000798µg/ml and these values are even higher than some obtained for untreated water.

Table 2. Oxytetracycline concentration in surface water

Sample name	**Sampling site characteristics**	**Mean concentration ± SD (µg/ml) n = 3**
Astra 1	Inside a storm drain	0.1484 ± 0.001891
Astra 2	Up stream	0.1386 ± 0.001002
Astra 3	Down stream	ND

Town 1 Town 2 Town 3	In a storm drain Up stream Down stream	0.1476 ± 0.002386 0.1532 ± 0.000133 ND
Aerodrome 1 Aerodrome 2	Up stream Down stream	0.1495 ± 0.001085 ND
Chipadze 1 Chipadze 2	Up stream Down stream	0.0708 ± 0.000305 ND
Chiwaridzo 1 Chiwaridzo 2	Up stream Down stream	0.0611 ± 0.000618 ND

Table 3. Oxytetracycline concentrations in treated drinking water

Sample name	**Mean concentration ± SD (µg/ml) n = 3**
Astra	ND
Town	ND
Aerodrome	ND
Main Campus	ND
Chipadze	0.0711 ± 0.000071
Cottco	ND
New Site	ND
Chiwaridzo	0.0660 ± 0.000798

DISCUSSION

This study was aimed at assessing levels of oxytetracycline in treated and untreated drinking water. The survey revealed that oxytetracycline was present in untreated water sources from Bindura. The antibiotic was detected frequently in storm drains and upper reaches of the stream and was not detected in the lower reaches. This may be due to dilution or complexation to sediment particles since oxytetracycline has been reported to complex readily to sediment particles. Presence of oxytetracycline in untreated water sources is worrisome since it may trigger microbial resistance or affect the self containing nature of aquatic ecosystem especially nutrient recycling (Zhou et al., 2011). Most of the treated water samples had no oxytetracycline detected. This may mean that the conventional methods used to treat water in Bindura are able to significantly retain and remove most of these

antibacterials. This can also be explained by adsorption of tetracyclines onto sediment. Adsorption studies carried out by (Agar, et al., 2005) showed that oxytetracycline has a low mobility in soil indicating high adsorption. More oxytetracyclines could have been left adsorbed onto soil and sediments which accounts for low values attained. (Simon, 2005) found out that oxytetracycline readily adsorbs onto sediment and loosely held molecules can also be desorbed back to the liquid phase by water currents. Therefore the sediment can act as a reservoir. Also organic matter present acts as a sorbent phase in soils for antibiotics. Chelation with metals between organic matter ligand groups and antibiotic ligand groups occurs as reported by (MacKay., et al 2005). Presence of oxytetracycline in Chipadze and Chiwaridzo drinking water samples is an anomaly and may be due to possible contamination of finished water with sewage as these sites are faced with serious water and sewer pipe bursts.

CONCLUSION

The present study results revealed that oxytetracycline is present in all surface water sources and some drinking water sources in Bindura indicating high levels of pharmaceutical pollution. There is a potential health risk on the environment and humans. There is risk of bacterial resistance and distruction of aquatic microflora. Presence of oxytetracycline in surface water calls for microbial resistance screening studies and development of pollution management regimes to prevent emission into the aquatic system.

ACKNOWLEDGEMENT

Authors would like to appreciate small equipment grant from DAAD and excellent technical assistance from University of Zimbabwe and Bindura University technicians. Thanks are also due to Mr Musodza GIS department for his kind help.

REFERENCES

1. Aga D., Connor O., Ensley S Payero., J Snow D., Tarkalson, D 2005. Journal of Agriculture Food Chemistry 53 (18), 7165–7171.
2. Batt A.L., Bruce I.B., Aga D.S., 2006. Evaluating the vulnerability of surface waters to antibiotic contamination from varying wastewater treatment plant discharges. Environmental. Pollution. 142, 295–302
3. Boxall B.A. B (2004). The environmental side effects of medication, European Molecular Biology Organization, 5(12): 1110–1116

4. Diwan V., Tamhankar A.J., Khandal R.K., Sen S, 2010. Antibiotics and antibiotic-resistant bacteria in waters associated with a hospital in Ujjain, India. BMC Public Health, 10,414
5. Franke AC., Berkhout ED., Iwuafor E.N.O., Nziguheba G., Dercon G., Vandeplas I., Diels J 2010. Does crop–livestock integration lead to improved crop production in the savanna of West Africa? Experimental Agriculture, 46, 439–455.
6. Gómez, M.J., Martínez Bueno, M.J., Lacorte, S., Fernández-Alba, A.R., Agüera, A.,
7. 2007. Pilot survey monitoring pharmaceuticals and related compounds in a sewage treatment plant located on the Mediterranean coast. Chemosphere, 66, 993–1002.
8. Halling-Sørensen, B., Nors Nielsen S., Lanzky PF., Ingerslev F., Holten L¨utzhøft H.C., Jørgensen S.E., 1998. Occurrence, fate and effects of pharmaceutical substances in the environment: A review. *Chemosphere*, 36(2), 357–393.
9. Jia A, Yang X, Jianying H, Asami M, Shoichi Kunikane (2009): Simultaneous determination of tetracyclines and their degradation products in environmental waters by liquid chromatography–electrospray tandem mass spectrometry Journal of Chromatography A, 1216, 4655–4662
10. Jodeh and Awartani., 2011. The Study of Fate and Mobility of Oxytetracycline and Doxycycline.*Jordan Journal of Chemistry* Vol. 6 No.3, 2011, pp. 347-360 in Soil Column Matrices
11. Kummerer K 2009. Antibiotics in the aquatic environment- a review- Part I**.** Chemosphere 75:417-434.
12. MacKay A., Canterbury M 2005. Journal Environmental Quality, 34, 1964-1971.
13. Navratilova P., Borkovcova I., Dračkova M., Janštova B., Vorlova L. 2009. Occurrence of tetracycline, chlortetracycline, and oxytetracycline residues in raw cow's milk. Czech Journal Food Science 27: 379–385.
14. R. Vallejo-Rodriguez., A. Lopez-Lopez., H. Saldarriaga-Norena., Mario Murillo- Tovar., Leonel Hernande-Mena 2011. Optimization of analytical conditions to determine Steroids and pharmaceutical drugs in water samples using solid phase extraction and HPLC, American Journal of Analytical Chemistry 2, 863-870.
15. Sanginga N., Dashiell K., Diels J., Vanlauwe B., Lyasse O., Carsky R.J., Tarawali S., Asafo-Adjei B., Menkir A., Schulz S., Singh B.B., Chikoye D., Keatinge D., Rodomiro O 2003. Sustainable resource management coupled to resilient germplasm to provide new intensive cereal–grain legume– livestock systems in the dry savanna. Agriculture, Ecosystems & Environment 100, 305–314.

16. Shafrir Michelle and Avisar Dror 2011. Development Method for Extracting and Analyzing Antibiotic and Hormone Residues from Treated Wastewater Sludge and Composted Biosolids. Water Air Soil Pollution. DOI 10.1007/s11270-011-1049-5
17. Sarmah A.K., Meyer M.T., Boxall A.B.A 2006. A global perspective on the use, sales, exposure pathways, occurrence, fate and effects of veterinary antibiotics (VAs) in the environment. Chemosphere 65, 725–759.
18. Simon N.S 2005. Loosely bound oxytetracycline in riverine sediments from two tributaries of the Chesapeake Bay. Environmental Science and Technology, 39, 3480-3487.
19. Sivri Nuket., Sandalli Cemal., Osman Birol Ozgumus., Feyza Colakoglu., Dilek Dogan 2012. Antibiotic Resistance Profiles of Enteric Bacteria Isolated from Kucukcekmece Lagoon (Istanbul–Turkey). Turkish Journal of Fisheries and Aquatic Sciences 12: 699-707.
20. Wang Q., Yates S.R 2008. Laboratory study of oxytetracycline degradation kinetics in animal manure and soil. Journal of Agricultural and Food Chemistry 56:1683–1688.
21. Wen Xianghua., Yannan Jia., Jiaxi Li 2009. Degradation of tetracycline and oxytetracycline by crude lignin peroxidase prepared from Phanerochaete chrysosporium – A white rot fungus**.** Department of Environmental Science and Engineering, Tsinghua University, Beijing 100084, PR China.
22. Yang J.F., Ying G.G., Zhao J.L., Tao R., Su H.C., Liu Y.S 2011. Spatial and seasonal trends of selected antibiotics in Pearl Rivers, South China. Journal of Environmental Science and Health, Part B, 46, 272-280.
23. Zhou Li-Jun., Guang-Guo Ying., Jian-Liang Zhao., Ji-Feng Yang., Li Wang., Bin Yang., Shan Liu 2011. Trends in the occurrence of human and veterinary antibiotics in the sediments of the Yellow River, Hai River and Liao River in northern China, Environmental Pollution 159, 1877-1885.