RESEARCH PAPER | Bio-Medical | Volume : 3 | Issue : 8 | Aug 2013 | ISSN - 2249-555X

Antibiotics in crops as influenced by irrigation with sewage effluent water: A case study

KEYWORDS

Antibiotics, tetracycline, oxytetracycline, sewage effluent, irrigation, soils, bioaccumulation, food chain Antibiotics, tetracycline, oxytetracycline, sewage effluent, irrigation, soils, bioaccumulation, food chain

O.P. Bansal

Chemistry Department, D.S. College, Aligarh-202001 (U.P.)

ABSTRACT *The release of pharmaceuticals particularly antibiotics into the aquatic environment and their occurrence in surface water, sewage and industrial effluents has become an increasing concern in recent years. In India, as there is a gradual decline in availability of fresh water for irrigation, the sewage and industrial effluents are being used for the irrigation of agricultural lands. A greenhouse experiment was undertaken to investigate the effect of soil properties and water quality on the uptake of two antibiotics viz. tetracycline (TC) and oxytetracycline (OTC) by tomato and cucumber plants. The data of this study denote that concentration of TC and OTC in fruits and leaves of both the plants were negatively correlated to soil organic matter content. The concentration of TC or OTC in roots and stems were relatively low and 71-82 % of antibiotics present in plants were accumulated in leaves. Bioaccumulation factor (Calculated as the ratio of antibiotics in plant to that in soil solution) in fruits was significantly positively correlated with soil organic matter; while concentration of antibiotics in soil solution was negatively correlated with soil organic matter. Greenhouse experiment using fresh water spiked with 1ug L-1 TC or OTC , sewage effluent and sewage effluent spiked with 1ug L-1 TC or OTC revealed that antibiotics can be taken up by plants and bioaccumulated from its background concentration in sewage effluent. The bioaccumulation factor for the fruits (0.81-1.18) was lower than for leaves (14.6-22.0). This study indicates the uptake of antibiotics by crops in organic matter poor soils irrigated with sewage effluent and highlights the potential risks associated with this agricultural practice.*

INTRODUCTION

In India, there is a gradual decline in availability of fresh water for agricultural fields; therefore, sewage and other industrial effluents are being used for irrigation of agricultural fields particularly in periurban areas. A number of researchers have reported the presence of active pharmaceutical compounds in the sewage effluent and waterways1-4. Pharmaceutical compounds are of potential concern as they are highly absorbable and can accumulate in soils, sediments and in tissues. Gu and Karthikeyan5 during their study found that 80% of wastewater influent and effluent contain tetracyclines (TC). TC is poorly adsorbed by human and animals after intake, about 50-80% are eventually excreted as unmetabolised parent compounds into domestic sewage. In the past decade, a number of workers have found TC in surface and ground water6-7. The accumulation of TC and other antibiotics in food chains via natural environments evokes concern of potential health risks from long –term low-level antibiotic exposure or biomagnifications, as low-level antibiotic exposure can cause bacteria to acquire and transmit antibiotics-resistant genes which potentially threatens ecosystem functions and human health. In recent years, several studies have shown that plants can take up antibiotics from the growth media when they are introduced either by spiking of the medium or irrigation water, or by sewage effluent application8-9 . The present study was conducted in order to analyze the uptake of tetracycline (TC) and oxytetracycline (OTC) by tomato and cucumber plants and its fractionation into edible and other plant organs as influenced by soil properties and quality of water (Fresh and sewage effluent)..

Materials and Methods

Three types of surface soils (0-30 cm depth), (Alluvial, Black cotton and peat) were collected from different parts of India. All the soils were air dried at room temperature and sieved by passing through a 100 mesh sieve. Physico-chemical characteristics like pH, organic carbon, and concentration of chloride, phosphate, total and DTPA extractable heavy metals viz., Zn, Mn, Cu, Ni, Cr, Pb, Fe and Cd were determined[10-11]. The values are given in Table 1.

Table 1: Physico- chemical characteristics of soils and water (mg L^{-1})

Characteristics	Alluvial soil	Black cotton soil	Peat soil	Fresh water	Sewage Effluent
pH	8.8	7.2	6.0	7.5	6.85
EC (dS m-1))	3.0	3.65	2.84	0.96	2.14
Organic matter content (%)	1.75±0.08	4.96±0.28	28.5±2.7	-	-
BOD (mg L-1)	-	-	-	2	58
COD (mg L-1)	-	-	-	4	200
Surface area (m2g-1)	48±5.1	115±8.1	88±15.4	______	____
Na (mg Kg-1)	545	368	334	12	36
K (mg Kg-1)	764	836	334	10	22
Zn (mg Kg-1)	0.25	0.35	0.30	0.25	1.32
Cr (mg Kg-1)	0.10	0.12	0.06	0.08	0.66
Pb (mg Kg-1)	0.08	0.10	0.12	0.10	0.40

Characteristics	Alluvial soil	Black cotton soil	Peat soil	Fresh water	Sewage Effluent
Cd (mg Kg-1)	0.02	0.02	0.04	0.04	0.16
Tetracycline (ug kg-1/ L-1)	nd	nd	nd	nd	2.5±1
Oxy tetracycline (ug kg-1/ L-1)	nd	nd	1.0±0.4	nd	8±2.5

Two experiments in several glazed earthenware pots were conducted in a greenhouse. In first experiment the effect of soil properties was studied. To examine the effect of soil properties, three soils with wide range of organic matter content and soil texture were used and irrigated with fresh water with or without (control) TC or OTC spiking 25 ug L^{-1}. The pots were filled with 5 kg of the soil four pre germinated seedlings of tomato (Lycopersicon esculentum) and cucumber were planted in each pot separately. The pots were irrigated daily to maintain the water holding capacity of the soil. These pots including blanks were also amended with NH_4NO_3; superphosphate and KCl. Ten replicate pots were used for each treatment.

In second experiment, to examine the effect of water quality the pots were irrigated by either fresh water or sewage effluent, with or without TC or OTC spiking 1 ug L-1. Major physico-chemical properties of irrigation water are given in Table 1. The sewage effluent was collected twice a week to minimize water alteration. Ten replicate pots were used for each treatment. The concentration of tetracycline and oxytetracycline in root, stem and edible parts of the crops were estimated.

Extraction of TCs

Soil samples: 1g of air dried soil sample was taken in a centrifuge tube, 15 ml of extraction buffer [0.1 M Mellvaine buffer (Na_2 HPO_4 and citric acid); 0.1 M EDTA and methanol 25:25:50 v/v] was added to it. The tubes were mixed with a vortex for 30 sec and then placed in an ultrasonic bath for 10 minutes, followed by centrifugation at 1200g for 15 min. The supernatant was decanted in to a glass bottle. The soil residues were extracted two more times and supernatants were combined and diluted to 400 mL with water, the pH of solution was adjusted to 2.8 using phosphoric acid. The antibiotics were extracted from the solution by solid phase extraction, using an Isolute strong anion exchange cartridge to remove interfering humic materials in tandem with a hydrophilic-lithophilic –balanced cartridge to extract the compounds. The cartridges were preconditioned with methanol and buffer, after washing the extracts were eluted with 2x1 mL of methanol. A flow rate of 10 mL min^{-1} was used for the extractions. The extracts were stored at -20^{0}c until analysis was complete.

Plant materials (stems, leaves, tomato, cucumber): 1-2.5g of air dried sample was taken in a centrifuge tube, 15 ml of extraction buffer [0.1 M Mellvaine buffer (Na_2 HPO_4 and citric acid); 0.1 M EDTA and methanol 25:25:50 v/v] was added to it. The tubes were mixed with a vortex for 30 sec and then placed in an ultrasonic bath for 10 minutes, followed by centrifugation at 1200g for 15 min. The supernatant was decanted in to a glass bottle. The soil residues were extracted two times more and supernatants were combined and diluted to 400 mL with water, the pH of solution was adjusted to 2.8 using phosphoric acid. Straw was crushed in a sieve –mill to particle length <1 mm. These particles were treated with Mellvaine buffer. Corn grains were grounded and sieved to mesh size fraction <0.1mm and treated with Mellvaine buffer.

The concentration of TC and OTC in soil and water extracts were analyzed by HPLC using an Agillent 1100 system with an octadecilsilan column (50mmx4mmx3um, AQ-YMC), TC and OTC were analyzed simultaneously. A gradient elution was carried out over 20 min with 0.1% formic acid in acetonitrile (Solvent A) and 0.1% formic acid in water (Solvent B). The initial percent of Solvent A was 5%, which was then increased to 30% from 0 to 7 min and remained at 30% from 7 to 8.5 min. The percentage of Solvent A was returned to 5% from 8.5 to 10 min and remained at 5% from 10 to 12

Table 2 Average Concentration of antibiotics in plants irrigated with fresh water spiked with 25 ug L-1 of TC or OTC.

Growth media	Tetracycline						Oxytetracycline					
	Tomato			Cucumber			Tomato			Cucumber		
	Soil solution (ug L-1)	Fruits (ug kg-1)	Bioaccumulation factor	Soil solution (ug L-1)	Fruits (ug kg-1)	Bioaccumulation factor	Soil solution (ug L-1)	Fruits (ug kg-1)	Bioaccumulation factor	Soil solution (ug L-1)	Fruits (ug kg-1)	Bioaccumulation factor
Alluvial Soil	12.12±0.5	22.6±2.9	1.86±0.4	12.52±0.6	23.1±2.3	1.84±0.5	11.2±0.8	20.8±2.9	1.86±0.4	11.34±0.6	19.8±2.0	1.91±0.3
Black Cotton Soil	4.15±0.3	15.2±1.1	3.66±0.9	4.75±0.4	15.8±1.5	3.32±1.0	3.64±0.5	13.2±1.4	3.62±0.7	3.18±0.3	12.2±1.0	3.84±0.8
Peat Soil	1.02±0.1	7.2±1.4	7.05±2.2	1.42±0.2	7.6±1.0	5.35±1.2	0.88±0.1	6.2±13	7.04±3.1	0.76±0.1	5.9±1.1	7.76±2.5

Volume : 3 | Issue : 8 | Aug 2013 | ISSN - 2249-555X

min. The flow rate was 0.70 mL min-1 throughout the analysis and simultaneous detection of TC and OTC was performed at 360 nm. Retention times of OTC and TC were 6.4 and 9.6 min. respectively. The minimum limit of detection was 0.5ug kg-1 soil.

All the chemicals used were of analytical grade.

Results and Discussion

Effect of soil properties; The results of uptake and bioaccumulation of antibiotics TC and OTC in the fruits of tomato and cucumber grown on three different soils irrigated by fresh water spiked with 25 ug L-1 antibiotics (TC or OTC) are given in Table 2. An examination of Table 2 denote that the concentrations of TC and OTC in tomato fruits were 22.6, 20.8; 15.2, 13.2; 7.2, 6.2 for alluvial, black cotton and peat soils respectively , while concentrations for cucumber fruits were 23.1, 19.8; 15.8, 12.2; 7.6, 5.9 ug kg^{-1} fresh weights respectively. The Concentrations of TC and OTC in the different soils' aqueous phase for tomato were 12.12, 11.2; 4.15, 3.64; 1.02, 0.88 and 12.52, 10.34; 4.75, 3.18 and 1.42, 0.76 ug L^{-1} for cucumber for alluvial, black cotton and peat soils respectively. These results denote that the concentration of antibiotics in the soils' aqueous phase was significantly negatively correlated with the level of organic matter in the soils[12] and amount of antibiotics taken by the plants was governed by the available TC or OTC in the soil solution which was in turn controlled by the soil-antibiotics interactions. The bioaccumulation factors for both the fruits for both studied antibiotics ranged from 1.84±0.5 to 1.91±0.3 for alluvial soil; 3.32±1.0 to 3.84±0.8 for black cotton soil and 5.35±1.2 to 7.76±2.5 for peat soil. The results also showed (Table 2) that the bioaccumulation factor for tomato fruits grown in presence of TC and OTC was almost same, while the bioaccumulation factor for cucumber fruit was higher in presence of OTC than TC. The results of study also denote that concentration of TC and OTC in both the types of fruits increased with time i.e. concentration of antibiotics in fruits developed at early stage were lower than the fruits developed at later stage, which may be due to increasing concentration of antibiotics in soil solution.

fresh water, non-spiked sewage effluent and spiked sewage effluent The uptake of antibiotics by plants in the sewage effluent irrigated system was lower than spiked fresh water irrigated sample, denoting an enhanced accumulation of TC or OTC in the solid phase of the soil. The antibiotics concentration in the fruits (ug kg^{-1} fresh weight) was similar to its concentration in the corresponding soil solution (ug L^{-1}). This relationship was also observed when the alluvial soil was irrigated with 25 ug L^{-1} antibiotics. In all the studied experiments the maximum concentration of antibiotics in all the parts of plants and soil solution was in the plants irrigated with the spiked sewage effluent water. The concentration of TC or OTC in cucumber plants were more than tomato plants (Table 3). The concentration of TC in both the plants part was more than OTC; it may be due to more sorption of OTC by soil than TC. The concentration of TC or OTC in leaves parts of both the plants grown on spiked sewage effluent irrigated soil was almost double than the plants grown on spiked fresh water, while in spiked sewage effluent irrigated plants the increase in the concentration of antibiotics in stem, root or fruits was 50-60% .The concentration of both the antibiotics in plants parts grown on non-spiked sewage effluent irrigated soils was lesser than in plants parts grown on spiked fresh water irrigated soils. This might be due to a binding of TC or OTC to dissolved organic matter[13], which reduces the free antibiotics concentration in the soil solution and uptake. The concentration of TC and OTC in the soil solution of the spiked fresh water irrigated soil increased from 0.80 ± 0.05, 0.75± 0.04 ug L^{-1}at the start of the experiment to 0.93± 0.06, 0.87± 0.06 respectively at the end of the experiment. The increase in antibiotics concentration may be due to sorption and accumulation in soil solid phase which in turn increases antibiotics concentration in soil solution with time. These inferences support our results regarding higher concentration of antibiotics in fruits that developed at the final stage of the growing period than that in fruits developed early during the study. The bioaccumulation factors calculated for the tomato and cucumber fruits in this study were 1.18 ± 0.28, 1.11± 0.18; 0.89± 0.16, 1.17± 0.22; 0.83± 0.26, 0.83± 0.16 for TC and 1.15 ± 0.24, 1.19± 0.30; 0.95± 0.14, 1.0± 0.1; 0.81± 0.22, 0.89± 0.18 for OTC for the plants irrigated with spiked fresh water, sewage effluent and spiked sewage effluent respectively, suggesting that the uptake potential of TC and OTC is mainly dependent on the available concentration in the soil solution and not on the concentration applied in the irrigation water. The bioaccumulation factors for the tomato and cucumber leaves were 15.4 ± 1.7, 19.2± 2.0; 17.4± 1.6, 22.0± 2.5; 14.2± 3.2, 15.3± 3.6 for TC and 15.3 ± 1.3, 16.1± 1.5; 16.7± 1.4, 18.0± 1.8; 12.4± 2.6, 14.8± 2.8, for OTC for the plants irrigated with spiked fresh water, sewage effluent and spiked sewage effluent respectively.

Table 3 Average Concentration of antibiotics in plants irrigated with fresh water spiked with 25 ug L-1 of TC or OTC.

Sample	Tomato			Cucumber		
	Spiked fresh water	Sewage effluent	Spiked Sewage effluent	Spiked fresh water	Sewage effluent	Spiked Sewage effluent
Irrigation water (ug L-1)	1.06	2.66±0.95	3.94±1.15	1.06	2.66±0.95	3.94±1.15
Soil solution (ug L-1)	0.93±0.06	1.12±0.14	2.08±0.08	0.90±0.05	1.02±0.16	2.28±0.12
Leaves (ug kg-1fresh biomass)	14.3±1.8	19.5±1.9	29.6±3.8	17.3±1.6	22.5±2.6	34.8±4.4
Stems (ug kg-1fresh biomass)	1.25±0.3	1.45±0.3	1.55±0.4	1.15±0.3	1.35±0.4	1.75±0.5
Roots (ug kg-1fresh biomass)	2.3±0.9	1.9±0.5	3.2±1.1	2.5±1.19	1.7±0.5	3.6±1.4
Fruits (ug kg-1fresh biomass)	1.1±0.9	1.0±0.5	1.6±0.6	1.0±0.7	1.2±0.6	1.8±0.6
Total uptake(%)	26	42	69	31	46	74

Tetracycline

Effect of water quality; The results of uptake of antibiotics TC and OTC by different parts of tomato and cucumber plants irrigated by 1ug L^{-1} spiked fresh water; sewage effluent and 1ug L^{-1} spiked sewage effluent grown on alluvial soil are given in Table 3. The preliminary results showed that the concentration of TC or OTC in roots leaves or stems grown in non-spiked fresh water were below detectable limit. The results showed that for both the plants the most of the concentrations of antibiotics accumulated in leaves, the concentration in the roots, fruits and stems were relatively low. The accumulations of TC and OTC in all the experiments, in the leaves were 71-82 % of the total applied antibiotics concentration, the bioaccumulation level in the roots and stems varied between 1.5 to 3.5%, and the total amount in the fruits was 12-20% of the total uptake in plants irrigated with spiked

Conclusions

Our studies show that antibiotics TC and OTC are taken up by tomato and cucumber plants when introduced to plants

Volume : 3 | Issue : 8 | Aug 2013 | ISSN - 2249-555X

via irrigation with spiked or non- spiked sewage effluent. Also denote that maximum uptake/ accumulation of antibiotics occur in the leaves. Our study indicates that introduction of antibiotics through the food-chain pathway is within the same magnitude or even higher than via drinking water. Therefore the combined effects should be investigated especially in areas using intensive irrigation of crops with sewage effluent. Further, the study causes a need for greater concern in crops like lettuce, whose edible parts are the leaves.

Acknowledgments
This research was supported by grants from UGC, New Delhi

REFERENCE
1. Kummerer, K. Antibiotics in the aquatic environment- a review –part I. Chemosphere | 75(2009a), 417-434. | 2. Segura, P.A., Francois, M., Gagon, C. and S. Sauve. Review of the occurrence of anti- | infectives in contaminated waste waters and natural and drinking waters. Environmental | Health Perspective 117 (2009), 675-684. | 3. Zhang ,Y. J., Geissen, S. U. and C. Gal. Carbamazepine and diclofenac: removal in | waste water treatment plants and occurrence in water bodies. Chemosphere 73(2008), | 1151-1161. | 4. Liu, H., Yang, Y., Kang, J., Fan, M. and J. Qu.Removal of tetracycline from water | by Fe- Mn binary oxide. Journal of Environmental Sciences 24(2012), 242-247 | 5. Gu, C. and K.G.Karthikeyan. Interaction of tetracycline with aluminium and iron | hydrous oxides. Environmental Science and Technology 39(2005), 2660-2667. | 6. Batt , A. L. and D. Aga. Simultaneous analysis of multiple classes of antibiotics by | ion trap LC/MS/Ms for assessing surface water and ground water contamination. | Analytical Chemistry 77 (2005), 2940-2947. | 7. Kummerer, K. Antibiotics in the aquatic environment- a review –part II. Chemosphere | 75 (2009b), 417-434. | 8. Kinney, C.A., Furlong, E.T.,Werner, S.L. and J.D.Cahill. Presence and distribution of | Waste water derived pharmaceuticals in soil irrigated with reclaimed water. | Environmental Toxicological Chemistry 25(2009), 317-326. | 9. Herklotz, P.A., Gyrung, P., Heuvel, B. V. and C.A.Kinney . Uptake of Human | pharmaceuticals by plants grown under hydroponic conditions. Chemosphere 78 (2010): | 1416- 1421. | 10. Bansal, O.P. Adsorption and Interaction of oxamyl and dimecron on some soils of | India. Journal Of Indian Society of Soil Science 30 (1982), 459-462. | 11. Lindsay, W.L. and W.A.Norvell. Development of DTPA soil tests for Zn, Fe, Mn | and Cu. Soil Science Society of America Journal 42 (1978), 421-428. | 12. Chefetz, B., Mualem, T. and J.Ben-Ari. Sorption and mobility of pharmaceutical | compounds in soil irrigated with reclaimed water. Chemosphere 73 (2008), 1335-1343. | 13. Maoz,A. and B.Chefetz. Sorption of the pharmaceuticals carbamazepine and | naproxen to dissolved organic matter: role of structural fractions. Water Research 44 (2010), 981-989.