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# SCREENING OF THE EFFECT OF GROUND WATER QUALITY ON THE STABILITY OF NORFLOXACIN AND DOXYCYCLINE IN DRINKING WATER OF POULTRY

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## ABSTRACT

This study was conducted to clarify the possible effects of ground water characteristics of the New Valley-Egypt, on the concentrations of both norfloxacin and doxycycline "*in- vitro*". Thirty two ground water samples (pooled samples) were collected and examined for their water quality parameters and heavy metals concentrations (pH, chloride ($Cl^-$), calcium ($Ca^{+2}$), magnesium ($Mg^{+2}$), sodium ($Na^+$), total hardness, electrical conductivity (EC), total dissolved solids (TDS), iron (Fe), copper (Cu), zinc (Zn) and nickel (Ni). Therapeutic doses of both norfloxacin and doxycycline were added separately to the ground-water samples and were left for different contact times. Results showed that norfloxacin concentrations significantly decreased with increasing the time of contact till 3 hours while, doxycycline showed a non-significant decrease which increased with increasing the contact time to 8 hours. Each of TDS, EC, $Mg^{+2}$, $Na^+$, $Cl^-$ and $Fe^{+2}$ showed significant positive correlations with the decreasing percent of norfloxacin while only $Ca^{+2}$ ions concentration showed a significant negative correlation with the decreasing percent of doxycycline.

***Key words**:* Groud-water- water quality, norfloxacin, doxycycline, poultry, heavy metals.

## INTRODUCTION

Poultry is one of the most widespread food industries worldwide where chicken is the most commonly farmed species. A large multiplicity of antibiotics are used to raise up poultry in most countries (Sahoo *et al.,* 2010; Landers *et al.,* 2012 and Boamah *et al.,* 2016). The main cause for adding antimicrobials in food-producing animals include prevention and control infections, promotion of growth and production improvement (Castanon *et al.,* 2007 and Mathew *et al.,* 2009). Both fluoroquinolones (FQs) and tetracyclines (TC) are among the most commonly used antibiotics in veterinary medicine in the world especially in poultry farms (Lindberg *et al.,* 2004).

Fluoroquinolones are broad-spectrum antibacterial agents with potent antibacterial activity against gram-positive and gram-negative bacteria (Chierentin and Salgado, 2015). Norfloxacin is rapidly bactericidal that it might have a great potential for treating common infections such as mycoplasmosis, colibacillosis and pasteurellosis in chickens, turkeys and geese (Gulkarov and Ziv, 1994). Tetracyclines exhibit their activity against a wide range of gram-positive and gram-negative bacteria, chlamydia, mycoplasmas, rickettsia and protozoan parasites (Roberts, 1996). In veterinary medicine, tetracyclines have been extensively used in the therapy of animal and poultry infections, prophylactic purposes and growth promotion (Levy, 1992).

A huge number of such antimicrobials that used in poultry production are reflected to be crucial in human medicine (WHO 2010 and WHO 2017). The undiscriminating use of such essential antimicrobials in animal production is prospective to hasten the development of antibiotic resistance in pathogens. This would lead to therapy failures, financial losses and could act as a source of gene pool for transmission to humans. Moreover, there are human health concerns about the existence of antibiotic residues in animal meat (Darwish *et al.,* 2013 and Aalipour *et al.,* 2013), eggs (Goetting *et al.,* 2011) and other edible animal products (Mehdizadeh *et al.,* 2010 and Addo *et al.,* 2011).

In recent years, enough confirmation highlighting the antimicrobial resistance from animals as a contributing factor for the inclusive burden of antimicrobial resistance and each of the extra

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unjustified overuse of antibiotics or the use of sub-therapeutic doses of antibiotics (Mathew *et al.*, 2007). Water that used as a substrate for antibiotic medication is a one of the important causes that may negatively affect a drug concentration in blood plasma of the treated birds. Low water quality was proven to decline antibiotics absorption" *in-vivo*" presenting sub-therapeutic doses than the required ones inside the animal bodies (Sumano *et al.*, 2004 and Kołodyńska *et al.*, 2012). Nevertheless, there are very scarce studies concerned with the possible in-vitro effect of water quality and characteristics on different antibiotic's solubility, stability and bioavailability.

## MATERIALS AND METHODS

1- **Area of study**

This study was conducted in Al-Dakhla Oasis - the New Valley, Egypt. Thirty two ground water samples were collected from different wells were situated around different poultry farms that depended mainly on ground-water for drinking of birds. The New Valley Governorate is situated 350 Km far from the Nile River, on the south western part of Egypt western desert. The sampling locations were given in (Fig. 1).

2- **Sampling**

Water collection and sampling were done according to APHA, 2005 in a representative amounts where 1L capacity dark glass bottles were used for sample's collection. All glass bottles were washed carefully with a final rinse with distilled water. All used bottles were sterilized before sampling. Groundwater samples were labeled and transported to the laboratory in an ice box for further analyses.


**Figure 1: Google map showing the localities of the sampling sites**

**3- Water analysis**

Collected ground-water samples were subjected to multiple analytical processes including the estimation of some of water quality parameters, heavy metals concentrations and antibiotic residues.

**3-i Estimation of water quality parameters**

Including the estimation of several chemical water characteristics as water pH, electrical conductivity (EC), some free ions including: chloride ($Cl^-$), calcium ions ($Ca^{+2}$), magnesium ions ($Mg^{+2}$) sodium ions ($Na^+$) and some of water complex molecules including: total hardness (T.H) and total dissolved solids (TDS).

Values of pH were estimated by using pH meter model JWNWAY 3505 (Eneji *et al.*, 2012). Electrical conductivity was estimated by conductivity meter (HI 9835- Italy). Total water hardness and total dissolved solids were estimated by using Lovibond Microprocessor Multi-direct Photometer- Germany. The analysis was conducted at Dept. of Animal Hygiene, Faculty of Veterinary Medicine, Assiut University. Chloride ions ($Cl^-$), Calcium ions ($Ca^{+2}$), magnesium ions ($Mg^{+2}$), sodium ions ($Na^+$) concentrations were estimated by Flame Photometer Model M 360 according to (Costanzo and Windhager, 1980) in laboratories of the Soil and Water Department, Faculty of Agriculture, Assiut University, Egypt.

**3-ii Estimation of heavy metals concentrations**

Water samples were subjected to cold and hot digestion processes using a mixture of $HNO_3$: $HClO_3$ (4:1 v/v) according to Chau and Lum, 1979 for the estimation of iron (Fe), copper (Cu), zinc (Zn) and nickel (Ni) A blank was prepared. calibration standards for all tested metals were prepared by serial dilution of concentrated stock solutions of 1000 mg/L and blank sample (deionized water) were analyzed for the digested samples according to Tewari and Singh, 2000 by using Flame Atomic Absorption Spectrophotometer (Perkin-Elmer Atomic Absorption Spectrophotometer model A Analyst 400). Analysis was done in triplicate and average values were calculated. Concentrations of the four analyzed heavy metals were calculated according to (Horwitz *et al.,* 2000).

**3-iii Estimation of antibiotics residues in ground-water samples**

Both norfloxacin (fluoroquinolones member) and doxycycline (tetracyclines member) residues in the all tested drinking water samples were estimated using Thin Layer Chromatography (TLC) in the Drug Researches Center, Assiut University according to Thangadurai *et al.* (2002).

**4- The experimental work:**

Norfloxacin 30% (1200 mg/L) and doxycycline 20% (300 mg/L) Pfizer Egypt-Pharma were used in the experiment. In the laboratory and in the same light condition and indoor temperature the therapeutic doses of both norfloxacin and doxycycline were separately added to the all tested water samples. Samples were left for 1 hour, 3 hours, 5 hours and 8 hours where, after each contact time the antibiotic concentration was re-evaluated. Control samples of both norfloxacin and doxycycline (which included the therapeutic dose of norfloxacin and doxycycline separately was dissolved in bi-distilled water) and measured in the same conditions after each separate contact time. All samples were estimated in triplicates. The antibiotic estimation was done by using the Thin Layer Chromatography (TLC) in the Drug Researches Center, at Assiut University according to Thangadurai *et al.* (2002).

**Technique used in Thin Layer Chromatography (TLC) for estimation of norfloxacin and doxycycline concentrations in all tested drinking water samples:**

This technique was used for both norfloxacin and doxycycline estimation either as a residue in the tested water samples or for measuring the therapeutic concentration after each contact time. Analysis was performed with several mobile phases for the individual identification of both fluoroquinolone and tetracycline (British Pharmacopoeia, 2012).

**i - Instrument specification:**

- CAMAG TLC scanner Ⅳ for scanning by absorbance and fluorescence, wavelength 190 – 900nm complete with deuterium lamp, tungsten – halogen lamp and mercury vapor lamp

- CAMAG Linomat 5, 230V, for sample application spot or band wise in quantitative and qualitative TLC. 100 µL Hamilton glass sample syringe for dosing of samples., Wincats version: 1.4.10, Scanning speed: 20mm/s, Data resolution: 100µm/step

- CAMAG Twin trough chamber for plates 20×10 and 10×10 cm with stainless steel lid.

**ii- Chromatographic preparation for antibiotics estimations:**

The selected mobile phase system (Methanol: Ammonia) was poured into the TLC tank lined with thick filter paper to help saturation of the tank. The tank was then covered with its lid and pre-saturated with the mobile phase system vapors for at least 30 minutes at room temperature (25° C) before use. Size of the plate used for the analysis is 20 ×10 cm. The samples were spotted in the form of bands 4mm length with CAMAG microliter syringe on the TLC plate using CAMAG Linomat 5. The slit dimensions were kept 4mm × 0.35mm, in the CAMAG scanner in reflectance absorbance mode.

The source of radiation was deuterium lamp emitting radiation in the range 190-900 nm. Scanning of the plate was carried out twice, at 280 and 378 nm for norfloxacine and doxycycline respectively.

**Statistical analysis:**

All data was analyzed using IBM SPSS Statistics for Windows version 25 (Field, 2013). Quantitative data were expressed as means ± standard deviation. Qualitative data were expressed as number and percentage. The nonparametric Mann–Whitney test, Friedman One-Way ANOVA test and Spearman's correlation were used for data which wasn't normally distributed. Independent Samples T test was used for normally distributed data. A 5% level was chosen as a level of significance.

## RESULTS

**Table 1:** The estimated values of water quality parameters and heavy metals concentrations in the tested groundwater samples.

| Water Parameters& Heavy metals | Minimum | Maximum | Mean± S.D. |
|---|---|---|---|
| **pH** | 5.00 | 6.00 | 5.6± 0.82 |
| **Total dissolved solids (T.D.S) (mg/L)** | 523.79 | 1987.53 | 1597.60±501.64 |
| **Electrical conductivity (EC) (dc/m)** | 0.85 | 3.55 | 2.49± 0.78 |
| **Total Hardness (T.H) (mg/L)** | 281 | 531 | 459.37± 83.38 |
| **Calcium ions ($Ca^{+2}$) (mg/L)** | 80 | 120 | 98.75± 18.85 |
| **Magnesium ions ($Mg^{+2}$) (mg/L)** | 195 | 420 | 360.62± 74.61 |
| **Sodium ions ($Na^{+}$) (mg/L)** | 72.90 | 290 | 194.11± 70.99 |
| **Chloride ions ($Cl^{-}$) (mg/L)** | 177.50 | 461.50 | 386.05± 106.40 |
| **Nickel (Ni) (mg/L)** | 0.0003 | 0.558 | 0.327 ± 0.175 |
| **Iron (Fe) (mg/L)** | 30.08 | 533.94 | 338.60± 174.04 |
| **Copper (Cu) (mg/L)** | 0.146 | 1.137 | 0.396± 0.311 |
| **Zinc (Zn) (mg/L)** | 4.88 | 13.95 | 10.21± 3.33 |
| **Norfloxacin residue** | 00 ± 00 | | |
| **Doxycycline residue** | 00 ± 00 | | |

**Table 2:** Statistical analysis of the impact of ground water on the therapeutic concentration of Norfloxacin and Doxycycline after different contact times.

| Type of Antibiotics | Control (Therapeutic dose) | Contact time / Decreasing % | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | after1 hour | | after 3 hours | | after 5 hours | | after 8 hours | |
| | | Mean ± S.D. | Decreasing % | Mean ±S.D. | Decreasing % | Mean ± S.D. | Decreasing % | Mean ±S.D. | Decreasing % |
| **Norfloxacin** | **1200 ± 0** | 1162.5 ± 1.05 | 3.13% | 1121.25 ± 1.98 | 6.56% | 1121.25 ± 1.98 | 6.56% | 1121.25 ± 1.98 | 6.56% |
| | **P-value** | 0.176 | | **0.038*** | | **0.038*** | | **0.038*** | |
| **Doxycycline** | **300 ± 0** | 297.5 ± 0.78 | 0.83% | 274.7 ± 0.98 | 8.42% | 268.7 ± 1.03 | 10.42% | 259.2 ± 1.06 | 13.58% |
| | **P-value** | 0.149 | | 0.144 | | 0.129 | | 0.110 | |

* P- value <0.05 is statistically significant

**Table 3:** Statistical correlations between water parameters& heavy metals concentrations and the decreasing % of both Norfloxacin and Doxycycline.

| Water Parameter | Norfloxacin | | Doxycycline | |
|---|---|---|---|---|
| | r | P-value | r | P-value |
| **pH** | 0.037 | 0.931 | 0.146 | 0.729 |
| **Total Dissolved Solids (mg/L)** | 0.712 | **0.048*** | 0.488 | 0.220 |
| **Electrical Conductivity (dc/m)** | 0.712 | **0.048*** | 0.488 | 0.220 |
| **Water Hardness (mg/L)** | 0.621 | 0.100 | - 0.078 | 0.854 |
| **Calcium ions ($Ca^{+2}$) (mg/L)** | - 0.052 | 0.903 | - 0.718 | **0.045*** |
| **Magnesium ions ($Mg^{+2}$) (mg/L)** | 0.921 | **0.001*** | 0.342 | 0.408 |
| **Sodium ions ($Na^{+}$) (mg/L)** | 0.951 | **0.000*** | 0.344 | 0.405 |
| **Chloride ($Cl^{-}$) (mg/L)** | 0.544 | **0.001*** | 0.226 | 0.590 |
| **Nickle(Ni) (mg/L)** | - 0.466 | 0.244 | - 0.342 | 0.408 |
| **Iron (Fe) (mg/L)** | 0.773 | **0.024*** | 0.098 | 0.818 |
| **Copper (Cu) (mg/L)** | 0.037 | 0.931 | - 0.123 | 0.772 |
| **Zinc (Zn) (mg/L)** | 0.503 | 0.204 | 0.537 | 0.170 |

* P- value <0.05 is statistically significant

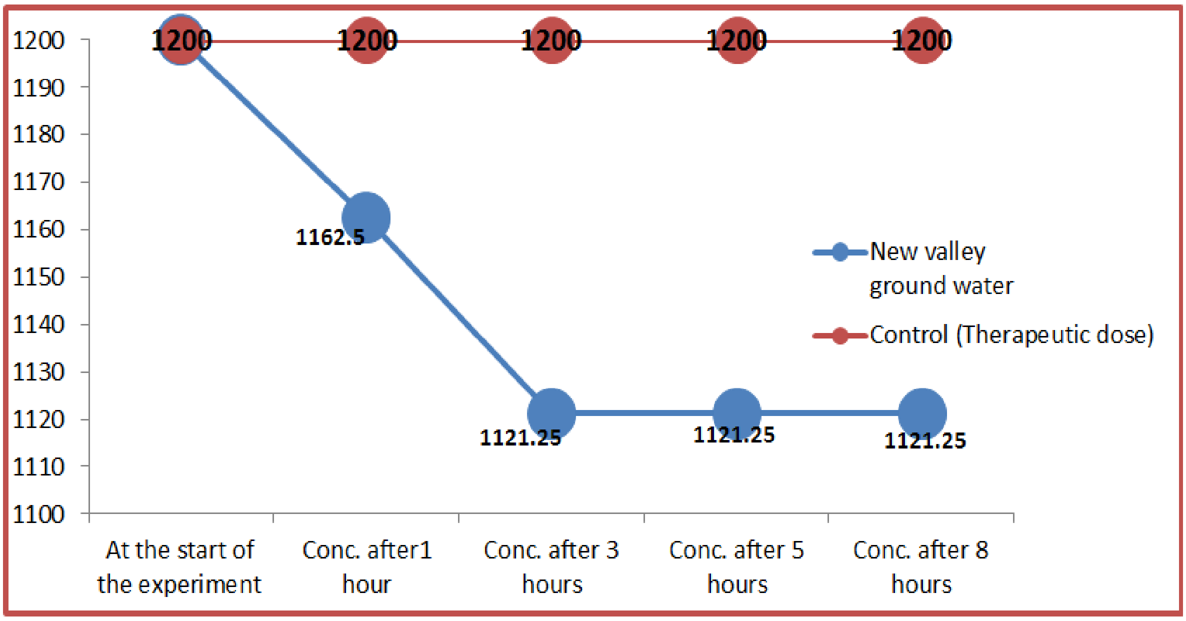


**Figure 2: Norfloxacin therapeutic concentrations in groundwater samples after different contact times.**

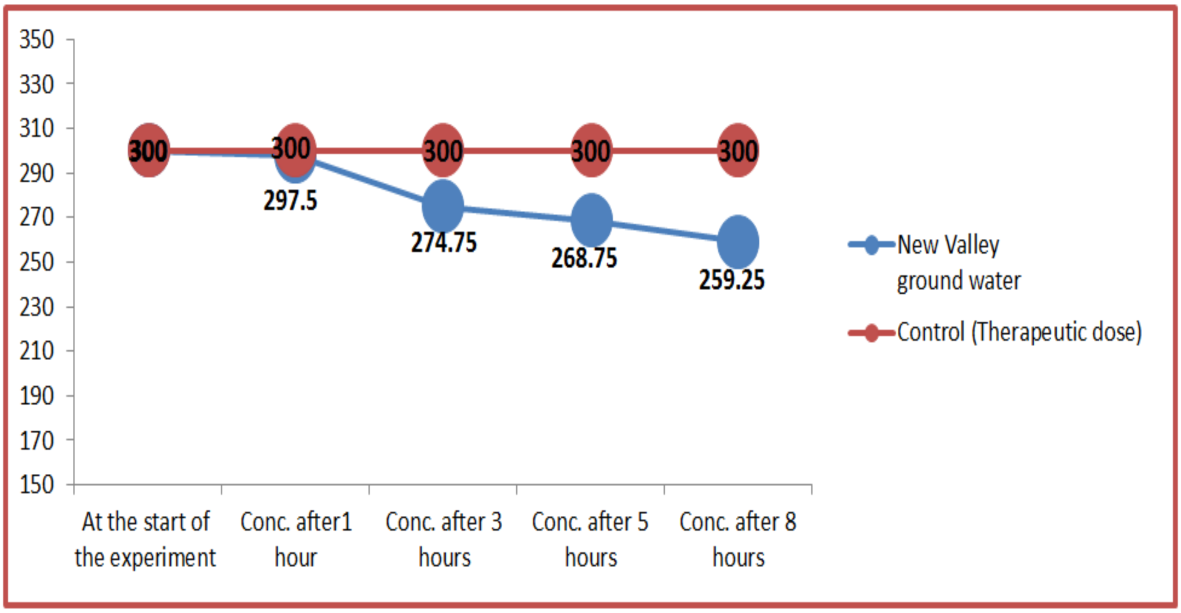


**Figure 3: Doxycycline therapeutic concentrations in ground water samples after different contact times.**

The estimated values of water quality parameters and heavy metals concentrations in the analyzed ground water samples were summarized in (table 1). It was clear that all pH values were recorded in an acidic range while all the other estimated water parameters showed variable degrees and concentrations. Iron among the analyzed heavy metals showed the highest concentrations while Nickel was recorded in the lowest concentrations. Residues of both norfloxacin and doxycycline were not detected in any of the analyzed groundwater samples.

Re-measuring of the therapeutic doses of the tested antibiotic concentrations in the examined water samples after each contact time was shown in (table 2). Norfloxacin concentrations gradually declined from the 1$^{st}$ till the 3$^{rd}$ hour of exposure time. No further decrease in norfloxacin concentration was recorded and it remained in a stable value after the 5$^{th}$ and 8$^{th}$ hour of contact time. The decreasing percent after the 1$^{st}$ hour (3.13%) was non-significantly different from the control value while, a significant difference ($P<0.05$) was recorded after the 3$^{rd}$ hour of contact time (6.65%). The control sample of norfloxacin did not show any decrease in its therapeutic concentration after all tested contact times (fig. 2).

Doxycycline showed relatively dissimilar manner from norfloxacin where, doxycycline concentration recorded a decrease after the 1$^{st}$ hour, with subsequent decreasing in its concentrations at the 3$^{rd}$, 5$^{th}$ and 8$^{th}$ hours of contact time (0.83%, 8.42%, 10.42% and 13.58%), respectively. After eight hours of contact time no an additional decrease in doxycycline concentration was recorded. None of the documented decreasing values of doxycycline concentrations was significantly different from the control value. The control samples of doxycycline did not show any decrease in its therapeutic concentration after all tested contact times (fig. 3).

The statistical correlation between water characteristics, heavy metals values and both norfloxacin and doxycycline were shown in (table 3). TDS, EC, $Mg^{+2}$, $Na^{+}$, $Cl^{-}$ and iron showed significant positive correlation with the decreasing percent of norfloxacin. On the other hand, only $Ca^{+2}$ ions concentration showed a significant negative correlation with the decreasing percent of doxycycline concentration.

## DISCUSSION

Comparing the obtained results of the estimated water quality parameters and heavy metals concentrations with the listed permissible limits of WHO, 2011, it was found that the mean values of $Ca^{+2}$ and $Na^{+}$ were within the permissible concentrations (200 mg/L) while both $Mg^{+2}$ and $Cl^{-}$ ions concentrations crossed the permissible limits (125 mg/L, 250 mg/L), respectively. All recorded pH values were lower than the optimum limits (6.5-8.5). Concerning heavy metals concentrations it was found that only copper was recorded within the permissible range (2 mg/L). Iron values were vastly higher than the permissible limits (0.3mg/L). In the same context both nickel and zinc exceeded the permissible limits (0.07 mg/L and 3 mg/L), respectively.

It was clear that application of norfloxacin and doxycycline in the ground water samples resulted in a drop in their therapeutic doses. In the same time and condition of the experimental work, the control samples of both norfloxacin and doxycycline did not show any decrease in their initial therapeutic concentrations. This finding ascertained that water characteristics definitely affected on the antibiotic's stability in water. This finding was in agreement with Lam *et al.* (2003); McIntyre and Lipman (2007).

Another stressing point is that the ground water characteristics did not affect the two tested antibiotics in the same degree and manner. Increasing the contact time of doxycycline in ground-water samples was parallel to its gradual non-significant decrease in its concentration. Although norfloxacin exhibited a limited effect by increasing the contact time which vanished after the 3rd hour of contact time, its declining in value was significantly different from the therapeutic control value of norfloxacin.

**Impacts of water quality characteristics on both norflxacin and doxycycline therapeutic concentrations "*in-vitro*"**

From the given results, it was obvious that, free ions concentrations, including $Mg^{+2}$, $Na^{+}$ and $Cl^{-}$ showed strong positive correlations with the decreasing values of norfloxacin. Moreover, another two related free ions parameters including TDS and EC showed the same effect. This mainly explained via the finding of Bečić *et al.*, 2014 who confirmed that fluoroquinolones had a very strong tendency to form complexes with ions. This finding concerning the possible effect of both $Cl^{-}$ and TDS on fluoroquinolones member degradation in water was in agreement with Lam *et al.* (2003), Fisher *et al.* (2006) and Ge *et al.* (2010).

Concerning heavy metals values only iron (Fe) showed a significant positive correlation with the decreasing percent of the therapeutic dose of norfloxacin in-vitro. This result was in agreement with Serafin and Stanczak (2009), who mentioned that norfloxacin reacted with iron at room temperature to form solid complexes.

Different outcome was obtained for doxycycline where, only $Ca^{+2}$ concentration recorded a negative significant correlation with its declining percent. None of the other estimated water quality parameters (pH, TDS, TH, $Mg^{+2}$, $Cl^{-}$ , $Na^{+2}$, Ec) showed any significant correlation with the decreasing values of doxycycline. The non-significant effect of the groundwater with an acidic nature on the decreasing percent of doxycycline concentrations was in agreement with (Marx, 2014) who found that there was no effect of the acidified water on the concentration of doxycycline after different contact times in- vitro.

Moreover, no significant correlations were recorded between all estimated heavy metals and doxycycline concentration. This result disagreed with Oka *et al.* (2000) who reported that tetracyclines tend to form strong complexes with copper and iron and Sunaric *et al.* (2009) who found that $Fe^{+2}$ and $Zn^{+2}$ ions had an ability to form stable complexes in aqueous solution with doxycycline and Andreu *et al.* (2016) who found that doxycycline had a potential interaction with the total content of nickel in different water samples.

## CONCLUSION

This study concluded that ground-water caused a decrease in both norfloxacin and doxycycline concentrations after different contact times. Norfloxacin showed no further decrease after the 3rd hour while doxycycline showed continuous insignificant decrease till the 8th hour of contact time. The free ions concentrations were remarkably correlated with norfloxacin drop in concentrations while only $Ca^{+2}$ ions were negatively correlated with doxycycline decreasing value "*in- vitro*". So, the process of adjusting the therapeutic dose of the administered antibiotic and the use of high quality water as a medium for bird medication is very important to avoid many subsequent problems including sub-therapeutic doses which result in drug resistance or failure of treating flocks by safe and effective types of antibiotics.

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# فحص تأثير جودة المياه الجوفيه على ثبات النورفلوكساسين والدوكسيسيكلين فى ماء شرب الدواجن

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أجريت هذه الدراسة بعد ازدياد الشكوى من المربيين والبيطريين من قلة فاعلية بعض المضادات الحيويه المستخدمه عن طريق اذابتها في مياه الشرب في كثير من مزارع الدواجن بمحافظة الوادي الجديد. وتهدف هذه الدراسه الى توضيح الآثار المحتملة للمياه الجوفية على تركيزات نوعين من المضادات الحيويه في المختبر. تم اختيار كل من النورفلوكساسين والدوكسيسيكلين لهذه الدراسه كما قد تم جمع عينات المياه من مجموعه من الابار والتي تستخدم مياهها في شرب الطيور والحيوانات. تم جمع عدد اثنين وثلاثين (٣٢) عينة مجمعه من المياه الجوفية وفحصها لتقدير بعض معايير جودة المياه وتركيزات المعادن الثقيلة (الرقم الهيدروجيني ، الكلوريد (CL) ، الكالسيوم ($Ca^{+2}$) ، المغنيسيوم ($Mg^{+2}$) ، الصوديوم ($Na^{+}$) ، عسر المياه ، التوصيل الكهربي (EC) ، والأملاح الكلية الذائبة (TDS) والحديد (Fe) والنحاس (Cu) والزنك (Zn) والنيكل (Ni) ، كما تم فحص متبقيات المضادات الحيويه في جميع عينات المياه. تم إضافة الجرعات العلاجية لكل من النورفلوكساسين والدوكسيسيكلين بشكل منفصل إلى عينات المياه الجوفية وتركت لأوقات تلامس مختلفه هي ساعه و٣ ساعات وخمس ساعات وثمانية ساعات على التوالي. كما تم تحضير عينات للتحكم وذلك في نفس الظروف من درجة حراره واضاءه. حيث كان يعاد قياس قيمة كل من النورفلوكساسين والدوكسيسيكلين بعد كل وقت من اوقات التلامس السابق ذكرها لتسجيل اي تغيير قد يحدث في تركيزاتها العلاجيه. وقد أظهرت النتائج أن تركيزات النورفلوكساسين انخفضت بشكل ملحوظ مع زيادة وقت التلامس حتى ٣ ساعات ، بينما أظهرت نتائج الدوكسيسيكلين انخفاضًا غير معنوي زاد مع زيادة وقت التلامس حتى ٨ ساعات .وعند دراسة العلاقه الاحصائيه بين بعض مكونات المياه التي قد تم قياسها ومعدل نقص تركيزات المضادات الحيويه. اظهرت النتائج ان كل من الاملاح الكليه الذائبه ، والتوصيل الكهربي ، وايونات الماغنيسيوم والصوديوم والكلور و وتركيز الحديد قد سجلوا ارتباطا معنويه إيجابيا مع انخفاض النسبة المئوية للنورفلوكساسين بينما أظهر تركيز أيونات الكالسيوم فقط ارتباطًا معنويا سلبيًا مع تناقص النسبة المئوية للدوكسيسيكلين. ونستخلص من هذه الدراسه انه يمكن ان يكون لبعض مكونات وخصائص مياه الشرب المستخدمه لاذابة المضادات الحيويه تأثيرا سلبيا على تركيزات المضادات الحيويه لمجرد اذابتها في هذه المياه وقبل ان تصل الى اجسام الطيوروبالتالي عدم وصول الجرعات العلاجيه بتركيزاتها المطلوبه الى بلازما الدم مما ينتج عنه عدم الحصول على النتائج المرجوة من استخدام تلك الادويه.