

Bioaccumulations of oxytetracycline and heavy metal on the earthworm *Aporrectodea longa*

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Impacts of antibiotic and heavy metals contaminations on agricultural soils attracted world attention. The present study aims to estimate the bioaccumulation of Oxytetracycline (OTC) and selected heavy metals (Fe, Cu, Zn) at Shandwil Island, Sohag, Egypt where they use the manure as an organic fertilizer. Concentrations of OTC, Cu, Fe and Zn in the earth worm (*Aporrectodea longa*) and the corresponding concentration in litter and soil at 25 locations at the studied site were determined. Some environmental factors (air/soil temperature, soil pH, soil water content and organic matter) were recorded at each investigated location. *A. longa* showed a significantly higher OTC concentration than litter and soil while studied heavy metals in soil showed higher concentrations than in earthworm and litter. Biomagnification (BMF) and bioconcentration (BCF) factors of OTC were 6.76 and 32.86, respectively. However, BMF and BCF of studied metals were relatively less than OTC and decreased in the following sequence Cu<Fe<Zn. Multiple regression analysis was used to show the effect of studied heavy metals and environmental factors on OTC concentration and bioaccumulation.

Keywords: Oxytetracycline, Heavy metals, Earthworm, Biomagnification, Bioconcentration.

INTRODUCTION

Earthworms are regarded as biological and physical engineers. They are main member in terrestrial invertebrates due to their role in preserving soil fertility. It is one of the sensitive indicators of soil quality and extensive used extensively in terrestrial ecotoxicology (Gao *et al.*, 2014). Although they play a vital role in soil formation as being so important creatures because of their usefulness to mankind and cultivation, they stayed un favorite as central point from scientists and researches for a long time.

Manure is used worldwide not only as a source of plant nutrients but also as a source of organic matter (Kumar *et al.*, 2005). Pharmaceuticals are released into the environment as a result of medical and veterinary usage (Puckowski *et al.*, 2016). Veterinary medicinal products (VMPs) are widely used to disease treatment, avoid infections, increase weight, or get better feed efficiency and keep the health of terrestrial and aquatic animals (Wang and yates 2008; Gao *et al.*, 2014; Gao *et al.*, 2015; Nica *et al.*, 2015; Li *et al.*, 2015; Jing *et al.*, 2015). Obviously, veterinary pharmaceuticals interact with soil minerals, organic matter, aqueous components, and organisms and are subject to sorption, photo hydrolysis, oxidation, and biodegradation. (Song, and Guo, 2014). Most veterinary antibiotics cannot be entirely absorbed by animals and are released through feces and urine as the parent compound or as metabolites (Gao *et al.*, 2015). The balance of the soil ecosystem may be destroyed as a result of antibiotic residues and their metabolites in soils. Oxytetracycline (OTC) is extensively preferred to animals in limited feeding process, Oxytetracycline is weakly metabolized in target animals and is excreted nearly in its parent structure (Salana *et al.*, 2013).

Heavy metal contamination has become a serious problem in the environment, due to the non-biodegradable property of heavy metals in soils, (Zheng *et al.*, 2013).

The bioaccumulation of trace elements in living organisms and biomagnification in them describes the processes and pathways of these (possible) pollutants from one trophic level to another. There are increasing concerns over the effects of veterinary antibiotics and heavy metals in agricultural soils (Kong *et al.*, 2006). Recently, extra attentions turn to the eco-toxicological effects of the antibiotic residues in the environment (Li *et al.*, 2015). Combined antibiotic and heavy metal pollution has received increasing worldwide attention (Gao *et al.*, 2015). A handful of studies have recently confirmed that the effects of veterinary antibiotics and heavy metals in soils is needed for improved soil risk assessment. Soil toxicity studies of several classical pollutants take earthworms as a model organism (Pino *et al.*, 2015).

These studies become more important to preserve the environment healthy. The environmental studies on ecotoxicological effects of

pharmaceuticals in soil ecosystems become more important to keep the environment healthy. Studies on the ecotoxicological effects of pharmaceuticals in soil biota are especially scarce (Pino *et al.*, 2015). As most studies on the pharmaceuticals occur in the laboratory and scarceness field study in this part; So the general idea for this research answer the question is the combination between heavy metals and pharmaceutical residues shape risk on the environment in the field. Therefore, the present study aims to determine the effect of OTC concentration and heavy metals on the earthworms as bio indicator for environmental health.

MATERIALS AND METHODS

Investigated area:

The present study was carried out in Shandwil Island (26°37' N, 31° 38' E), Sohag governorate, Egypt (Fig. 1). This investigated area is a research farmland characterized by many types of different plants such as alfalfa, fenugreek, wheat, palm, Mangoes, citrus and banana. Animal manures are used as fertilizers for this research area. Citrus orchard (about 100Acre) was chosen for this study, where recorded the high abundance of earthworm.

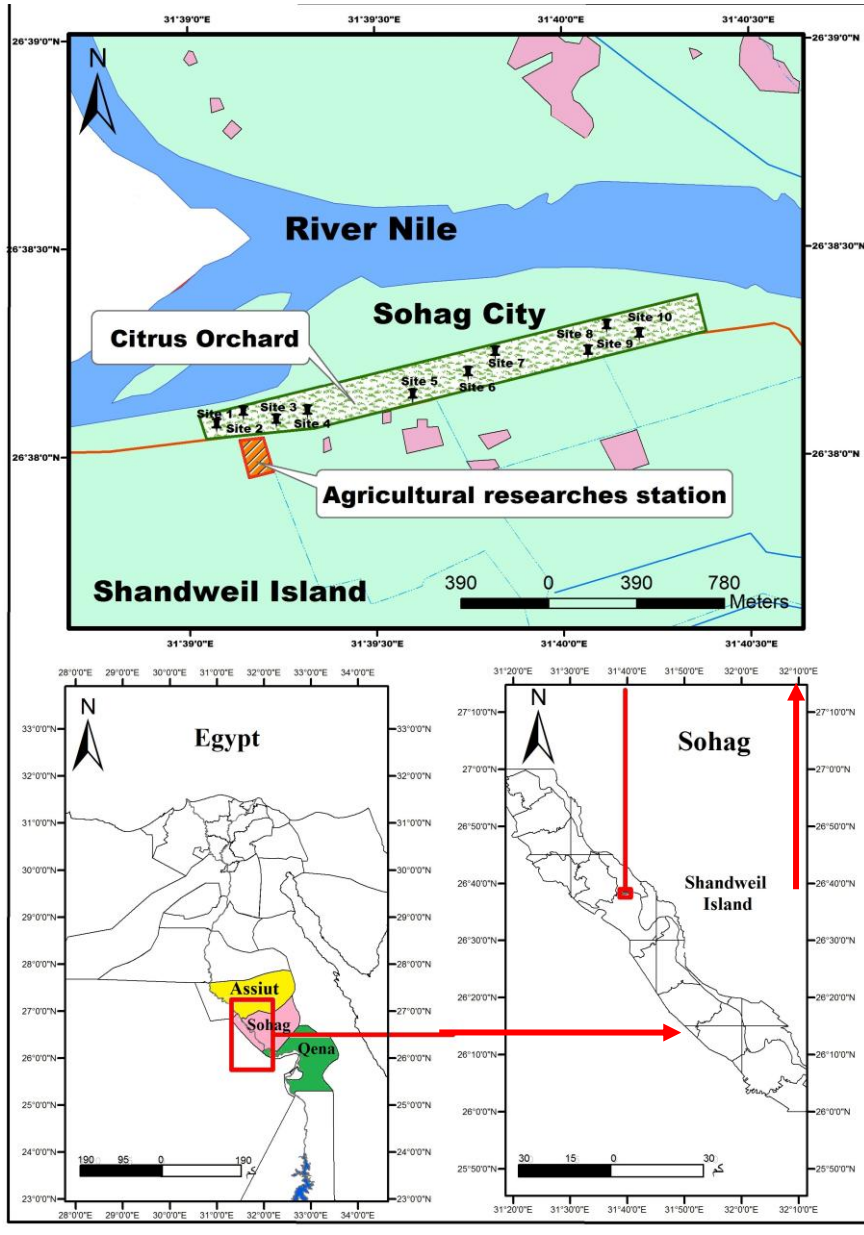


Fig 1. Map showing site of collection area Shandweil Island, Sohag governorate, Egypt.

Sampling

Samples were collected randomly during February 2016 from 25 locations, between each location and the other about 10-15 meters. Earthworms were handily collected from the soil till depth 20 cm. from

each location samples from litter and soil were sampled. The collected samples of earthworm, litter (humus) and soil samples were labeled and preserved for transport to the Lab., where soil and litter samples stored in plastic bags and earthworms stored in plastic boxes. In Lab. earth worm *Aporrectodea longa* was separated and preserved in -20°C till analyses.

Measurements of ecological factors

During sampling, air temperature one meter above soil surface, and soil temperature were recorded, using a normal thermometer. In Lab, soil pH was measured using suspension of soil and distilled water at ratio of 1:2, then it was measured by pH-meter. Water content was determined by relating the water loss of 50 g soil sample to the dry weight (at 110°C). The weight loss of 5 g dry soil sample after 8 h in Muffle furnace at 500°C was used to calculate the organic matter content, which was expressed as percentage of the original dry weight of the sample.

Preparation of samples for chemical analysis

For Oxytetracycline (OTC) analyses, samples of soil (0.51 ± 0.003 ; Mean \pm SD), litter (0.5 ± 0.001 ; Mean \pm SD), and earthworm (0.11 ± 0.04 ; Mean \pm SD) were prepared from each collected location. 5ml of HCl was added to each sample. Then they covered in dark place as the temperature and light affect the OTC concentration. Samples were filtrated in measuring flask and distilled water was added to 25 ml then preserved till measuring time.

For heavy metals; 10 ml of (100 ml HNO_3 +300 ml of HCl) was added to specific weight of soil (0.5 ± 0.002 ; Mean \pm SD), litter (0.32 ± 0.01 ; Mean \pm SD), and earthworm (0.11 ± 0.04 ; Mean \pm SD) from each investigated location. After digestion, each solution was filtrated in measuring flask and distilled water was added to 100 ml then preserved in 4°C till measuring time.

Chemical analysis

UV – spectrophotometer was used to estimate OTC concentration. Both multi and fixed Wavelength methods have been used to determine the desired compound(s).

The heavy metal concentrations were analyzed using Perkin Elmer equipped A Analyst 400 atomic absorption spectrophotometer with

protective coating. Deuterium background corrector and two built-in EDL power supplies.

OTC was measured using Genway spectrophotometer at wavelength of 430 cm^{-1} that have been reported for OTC detection (Al warthan *et al.*, 1991).

Statistical analysis:

Regarding to Bioaccumulation patterns, the bioconcentration factors (BCF) (indicating the ratio of metal/OTC concentration in earthworm to soil metal/OTC concentration) and the biomagnifications factors (BMF) (indicating the ratio of metal/OTC concentration in earthworm to litter metal/OTC concentration) were calculated for studied heavy metals and OTC for the common earthworm *A. longa*. Analysis of Variance on SPSS software package (version 20) (SYSTAT statistical program) was used to test the present data. Duncan test was used to detect the distinct variances between means.

Pearson correlation and stepwise multiple regression were used to investigate relationships between OTC Bioaccumulation with environmental factors and metal/OTC concentrations.

RESULTS

Table (1) shows the mean values of selected physical parameters including; air and soil temperature, pH, Humidity, organic matter, number of *A. longa*, Density of *A. longa*. The calculated mean \pm SD results of the physical parameters are as follow: Number of *A. longa* (6.76 ± 4.54), the density (0.61 ± 0.41), air temperature (18.82 ± 0.82) soil temperature (14.10 ± 0.76), humidity % (20.53 ± 3.87), pH (8.58 ± 0.25). Table (2) displays the comparison between mean concentrations of OTC, Fe, Cu and Zn in soil, litter and *A. longa* at different sites. Moreover, bioaccumulation factor divided into biomagnification factor (BMF) (indicating the ratio of metal concentration in *A. longa* to litter metal concentration) and Bioconcentration factor (BCF) (indicating the ratio of metal concentration in *A. longa* to soil metal concentration) were calculated for *A. longa* samples in each site (Table 3).

Oxytetracycline:(OTC)

OTC is susceptible to microbial transformation as well as abiotic processes such as hydrolysis and photolysis. Tracking the OTC concentration in the samples showed that the mean concentration in *A. longa* ranged from the lowest value 472.29 µg/g and the highest value 3419.75 µg/g. In litter, it ranged from 16.73 µg/g and the highest value 568.35 µg/g. While in soil, ranged from 3.84 µg/g and the highest value 275.97 µg/g. There were no significance between animals OTC conc. and OTC conc. in litter and soil. OTC BMF ranged between the lowest value 1.26 µg/g and the highest value 33.30 µg/g. Cu BCF ranged between the lowest value 5.39 µg/g and the highest value 351.42 µg/g. Statistical tests showed that *A. Longa* OTC concentrations were highly significantly differences ($p < 0.01$) with soil Cu, Zn and Fe concentrations. Generally, OTC levels in animals, litter and soil were higher than Fe, Zn and Cu levels. Stepwise multiple regression showed that *A. longa* OTC conc. is affected by Zn soil concentration. The model equation is;

$$\text{OTC } A. \text{ longa conc.} = 992.5 + 0.5 \text{ Zn soil conc.}$$

Litter OTC conc. is affected by OTC BMF. The model equation is;

$$\text{Litter OTC conc.} = 471.3 + 0.87 \text{ OTC BMF}$$

Soil OTC conc. is affected by Fe, and Cu soil concentrations. The model equation is;

$$\text{Soil OTC conc.} = -55.5 + 0.5 \text{ Cu soil conc.} + 0.4 \text{ Fe soil conc.}$$

Iron:(Fe)

At the 25 sites, Fe concentration were higher as compared with the other metals in the soil, litter and *A. longa*. Fe mean concentrations ranged between the lowest value 65.57 µg/g and the highest value 10175.47 µg/g. In litter, it ranged between the 60.77 µg/g and the 776.65 µg/g. In soil, it ranged between the 569.16 µg/g and the 2009.11 µg/g.

A. Longa Fe concentrations were highly significantly different ($p < 0.01$) with Cu concentration in the litter. They were higher than soil and litter Fe concentrations. Statistical tests showed that there were significant differences between Fe conc. in *A. longa* with those in litter and OTC conc. In litter, whereas no significant difference between Fe level in animal and in soil, there were highly significant differences between Fe level in soil and litter.

Fe BMF and Fe BCF were highly significant with Fe level in animal. No correlation exists between animal Fe conc. and Zn conc. in animal, litter, soil and environmental factors. Stepwise multiple regression shows that *A. longa* Fe conc. is affected by Cu litter conc., Fe BCF and Fe BMF. The model equation is;

$$\text{Fe conc. } A. \text{ longa} = 153.9 - 0.12 \text{ Cu litter conc.} + 0.6 \text{ Fe BCF} + 0.5 \text{ Fe BMF}$$

Litter Fe conc. is affected by *A. longa* Fe conc. and soil Zn concentration. The model equation is;

$$\text{Litter Fe conc.} = 678 - 0.5 \text{ Fe conc. } A. \text{ longa} + 0.4 \text{ Zn soil concentration}$$

Soil Fe conc. is affected by soil Zn concentration. The model equation is;

$$\text{Soil Fe conc.} = 544.4 + 0.7 \text{ Zn conc. soil}$$

Copper:(Cu)

Copper belongs to the substances which are essential for human health, e.g. by being part of enzymes involved in specific metabolic processes. However, it may be harmful in higher doses by causing gastrointestinal distress, damage to liver, the immune system, neurological system and reproductive ability.

Cu concentrations in *A. longa* ranged between the lowest value 0.96 µg/g and the highest value 14.68 µg/g. In litter, it ranged between the 1.64 µg/g and the 15.30 µg/g. In soil, it ranged between the 27.14 µg/g and the 104.74 µg/g. there were no significant difference between the animal and litter Cu concentrations. It is clear that cu levels in animal are lower than litter, and soil.

Statistical tests showed that *A. Longa* Cu concentrations were highly significant differences ($p < 0.01$) with *A. longa* Zn concentration. There were significant differences between *A. longa* Cu concentration and litter Zn concentration. Generally, Cu levels in animals, litter and soil were lowest compared with Fe, and Zn. There were highly significant differences between *A. longa* Cu BMF and Cu BCF. Cu BMF ranged between the lowest value 0.13 µg/g and the highest value 2.44 µg/g. Cu BCF ranged between the lowest value 0.02 µg/g and the highest value 0.28 µg/g. No correlation exists between animal Cu conc. and Fe in animal, litter, soil and environmental factors. Stepwise multiple regression shows that *A.*

longa Cu conc. is affected by Zn *A. longa* conc., Cu BMF, Cu BCF, the model equation is;

$$A. longa \text{ Cu conc.} = -0.7 + 0.72 \text{ Zn } A. longa \text{ conc.}$$

$$A. longa \text{ Cu conc.} = -0.1 + 0.52 \text{ Cu BMF} + 0.5 \text{ Cu BCF}$$

Litter Cu conc. is affected by litter Fe conc. and Fe BCF. The model equation is;

$$\text{Litter Cu conc.} = 2.7 + 0.75 \text{ Fe conc. litter} + 0.3 \text{ Fe BCF}$$

Soil Cu conc. is affected by OTC soil concentration and Zn litter. The model equation is;

$$\text{Soil Cu conc.} = 30.2 + 0.6 \text{ OTC soil concentration} + 0.4 \text{ Zn conc. litter}$$

Zinc: (Zn)

The mean Zn conc. in animals ranged from 2.62 $\mu\text{g/g}$ and the highest value 302.72 $\mu\text{g/g}$. In litter, it ranged from 2.89 $\mu\text{g/g}$ and the highest value 281.27 $\mu\text{g/g}$. In soil, ranged from 27.14 $\mu\text{g/g}$ and the highest value 150.94 $\mu\text{g/g}$. There were no significant differences between Zn concentration in animals and Zn concentration in litter and soil. Zn BMF ranged between the lowest value 0.39 $\mu\text{g/g}$ and the highest value 21.55 $\mu\text{g/g}$. Cu BCF ranged between the lowest value 0.02 $\mu\text{g/g}$ and the highest value 3.25 $\mu\text{g/g}$. Statistical tests showed that *A. longa* Zn concentrations recorded highly significant differences ($p < 0.01$) with animals Cu conc. and organic matter. There were significant differences between *A. longa* Zn concentration and soil pH. Generally, Zn levels in animals, litter and soil were lower than Fe, while Zn levels were higher than Cu level. There were highly significant differences between *A. longa* Zn conc. And Cu BMF, Cu BCF, Zn BCF. Stepwise multiple regression showed that *A. longa* Zn conc. is affected by Cu BMF, Zn BCF, the model equation is;

$$\text{Zn } A. longa \text{ conc.} = 3.2 + 0.7 \text{ Zn BCF} + 0.4 \text{ Cu BMF}$$

Litter Zn conc. is affected by OTC BMF, Cu soil conc., The model equation is;

$$\text{Litter Zn conc.} = -51.8 + 0.5 \text{ OTC BMF} + 0.4 \text{ Cu Soil conc.}$$

Soil Zn conc. is affected by Fe soil conc., Cu soil conc., The model equation is;

$$\text{soil Zn conc.} = -19.3 + 0.6 \text{ Fe soil conc.} + 0.4 \text{ Cu soil conc.}$$

Table 1: Calculated mean, standard deviation, minimum and maximum values of ecological factors and *A. longa* density.

	Sp richness	No. of earthworm Aporetodea longa	Density of <i>A. longa</i>	air temperature	soil temperature	Humidity %	Organic matter %	pH
Mean	3.12	6.76	0.61	18.82	14.1	20.53	7.46	8.58
SD	0.6	4.54	0.41	0.82	0.76	3.87	1.21	0.25
Maximum	4	15	1.35	20.3	15.9	27.9	10.3	9
Minimum	2	1	0.09	17.3	12.6	14.1	5.5	8.1

Table 2: Mean, standard deviation, minimum and maximum values of OTC, Fe, Cu and Zn Concentrations.

	OTC µg/g (ppm)			Fe µg/g (ppm)			Cu µg/g (ppm)			Zn µg/g (ppm)		
	soil	litter	<i>A. longa</i>	Soil	litter	<i>A. longa</i>	Soil	litter	<i>A. longa</i>	Soil	litter	<i>A. longa</i>
Mean	110.35	383	1686	1138	378	733.9	50.82	9.41	2.58	67	28.94	71.9
SD	70.96	133	726.3	475.1	209	2171	18.52	3.25	4.73	37	55.6	74.7
Maximum	275.97	568	3420	2009	777	10175	104.7	15.3	14.68	151	281.3	303
Minimum	3.84	16.7	472.3	569.2	61	65.57	27.14	1.64	0.96	27	2.89	2.62

Table 3: Mean values variations of bioconcentration factors (BCF) and biomagnifications factors (BMF) for OTC, Fe, Cu and Zn in *Aporrectodea longa*.

	OTC		Fe		Cu		Zn	
	BCF	BMF	BCF	BMF	BCF	BMF	BCF	BMF
Mean	32.9	6.8	0.8	2.5	0	0.3	0.9	3.41
SD	67.7	8.2	2.9	7.1	0	0.63	1	5.45
Maximum	351	33	15	35	0	2.44	3.3	21.55
Minimum	5.39	1.3	0	0.1	0	0.13	0	0.39

DISCUSSION

Accumulation of iron, and copper in soils is mainly due to anthropogenic origin, such as mining or industrial activities, Agricultural use of products containing copper is also common, especially in pesticides applied in vine yards and orchards (Fishel,

2014). This might be a reason, why soil samples with high Fe, and Cu concentrations were found. In contrast Zn ions could be absorbed easily by plant roots and animal cell membrane forming complexes with protein structures. These facts explain the high concentration of Fe and Cu in soil rather than the high concentration of Zn in litter and *A. longa*. In the other hand the low concentration of OTC in soil and litter compared with that in *A. longa* is due to the physical characters of OTC itself. It is known that OTC is easily decomposed under temperature and light effect. Both ecological parameters can be observed in both plant (photolysis process) and soil (heat absorption and emission).

The small BMF for OTC compared with BCF suggested that oxytetracycline was accumulated gradually in *A. longa* tissue through feeding and could not be decreased due to the absence of degradation conditions.

Oxytetracycline (OTC):

Oxytetracycline (OTC) is a member of the tetracycline family of antibiotics, widely used for therapeutic purposes in humans as well as an antibiotic and growth promoter in animal farming.

The present results indicated that the order of OTC concentration was, *A. longa* > litter > soil, this is can be attributed to the effect of ecological factors of light and temperature. More specific, the OTC concentration in soil was found to be 25% and 10% than the concentration in litter and *A. longa*, respectively. The reason is due to the direct contact of soil to air which causes the decomposition of OTC, while for OTC in litter, the photolysis process may also lead to the decomposition of OTC. Only *A. longa* has the indirect contact to light and temperature effect lead to the increase of OTC concentration in *A. longa* rather than in litter and soil.

This process is quantified by the bioconcentration factor (BCF), the ratio of chemical concentration in an organism to that in the ambient environment. In contrast, bioaccumulation is accumulation from all sources, both ambient and dietary (Arnot and Gobas, 2006). The biomagnification factor (BMF) is the ratio of a chemical's concentration in an organism to that in its diet.

At present, there are growing concerns regarding the ecological/environmental risks associated with OTC exposure. Given its antibacterial properties, development of antibiotic-resistant bacteria in humans, and the environment in general, has been cited as an

obvious risk with use. The number of studies on human risk is relatively small. Many factors can affect the bioavailability of OTC in the aquatic food chain. Divalent cations (e.g. Ca^{2+} and Mg^{2+}) in water can bind to OTC and form relatively stable complexes that reduce gastrointestinal absorption efficiency (Sassman and Lee, 2005). Environmental factors such as temperature, pH and light intensity can affect the degradation of OTC in water and sediment (Burhenne *et al.*, 1997; Boonsaner and hawker, 2013).

Heavy metals

Heavy metals concentrations in the tissues of the earthworm species collected from the studied area were generally high. Fe ions are known to be partially soluble, which means that Fe tends to precipitate with the small increasing in concentration as well as the presence of organic pollutants such as OTC. This explains the obtained order of the Fe Concentration as Soil > *A. longa* > litter.

Zn bound to one or more of the animal's enzymes which give good explanation of the high Zn Concentration in *A. longa* rather than in soil and litter.

OTC may interact with Fe, Cu and Zn ions through coordination and adsorption on their insoluble salts. The chemical interaction between OTC and Fe, Cu and Zn either by coordination or adsorption could protect OTC molecules from decomposition by heat or light.

Earthworms uptake OTC from soil and recycle it through hydrolysis and decay, then it interacts with Fe, Cu, and Zn ions through coordination as well as adsorption, which give indication to the significant correlation between Fe concentration in soil and OTC in *A. longa* and soil. This explains the significant correlation between OTC concentration in soil and *A. longa* with Fe, Cu and Zn concentration in soil.

The significant correlation between OTC concentration and Cu^{2+} can be explained using the previous reported results about the complex formed between Cu ions and OTC, which tends to have square planar geometry with Cu^{2+} : OTC ratio of 2:1. This ratio elucidates that Cu^{2+} will strongly chelate to OTC in their coexisted system, also it was reported that the presence of Cu^{2+} typically inhibited the direct photolysis rate of OTC in neutral pH condition. Jin *et al.*, 2016

It is known that OTC occurs primarily as zwitterionic and negatively charged forms under nearly neutral condition, the antibiotic binds to Cu^{2+} ion via: (1) the oxygen atom of enol form hydroxyl group at site 3 and the nitrogen atom of dimethylamino group, (2) the oxygen atoms of hydroxyl group at site 12 and carbonyl group at site 11, or (3) the oxygen atoms of hydroxyl group at site 10 and carbonyl group at site 11, thereby forming at least five speciation (Zhang *et al.*, 2012).

The threshold of complete antibiotic removal of OTC is 40 ppm. In the present study, the concentration of OTC is 2.5 higher than the threshold value. For heavy metals (Fe, Cu, Zn), the concentration under the threshold value.

The present study confirmed to there is a necessary to treatment before using the manure as a fertilizer. In order to protect the environment, the pollution that is due to the presence of pharmaceutical residues.

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التراكم الحيوي للأوكسي تيتراسيكلين والمعادن الثقيلة لدودة الأرض أبروكتيديا لونجا

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ان تلوث الاراضى الزراعية بالمضادات الحيوية والعناصر الثقيلة من اهم المشاكل التى تلتفت انتباه العالم. لذلك دراستنا الحالية تهدف لتقدير التراكم الحيوي لاحد المضادات الحيوية واكثرها انتشارا واستخداما وهو ال أوكسي تيتراسيكلين وتأثيره بوجود بعض العناصر الثقيلة(الحديد- النحاس- الزنك) . وقد أجريت تلك الدراسة فى مزرعة البحوث الزراعية بجزيرة شنديول والتي تقع شمال محافظة سوهاج حيث انها تستخدم المخلفات الحيوانية كمخصبات عضوية. ولفهم التراكم الحيوي لابد من تقدير تركيز كل من أوكسي

تيتراسيكلين والحديد والزنك والنحاس في ديدان الأرض باعتبارها كائن نموذجي لمراقبة التلوث البيئي وعمل مقارنة بين تلك التركيزات في كل من التربة والدوبال وديدان الأرض في ٢٥ موقع موزعين عشوائيا في المزرعة محل الدراسة مع التسجيل بعض العوامل البيئية مثل (درجة حرارة كل من الهواء والتربة- نسبة الرطوبة-PH) في كل المواقع محل الدراسة.

سجل أوكسي تيتراسيكلين أعلى تركيز في الديدان يليه الدوبال ثم التربة في حين أن العناصر الثقيلة سجلت أعلى تركيز في التربة يليه الدوبال ثم في الديدان. وتم استخدام أحد تحليل الانحدار المتعدد لتوضيح تأثير العناصر الثقيلة والعوامل البيئية على تركيز أوكسي تيتراسيكلين وتراكمه الحيوي في البيئة