Effect of Spiked Soils with Aflatoxin on Its Sorption and Bioavailability

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ABSTRACT

The study was conducted to elucidate the sorption and bioavailability of aflatoxins mixture (B1, B2, G1, and G2) in two soils (clayey and sandy) differed in physicochemical properties under (un)treated soil with manure or urea fertilizer. Sorption and greenhouse experiments were designed for this purpose. Sorption isotherms were obtained using the batch equilibrium technique. The sorption isotherms fit the Freundlich adsorption equation according to the high R^2 determination coefficients. In clayey soil, there was not detected aflatoxin concentration in soil-aqueous phase which referred to high sorption of aflatoxin in soil-solid phase, and all the aflatoxin disappearance from solution was referred to sorption reaction not for biodegradation. The high content of clay fraction in this soil could be the reason for this high sorption capacity. In sandy soil, the sorption isotherm data indicated high retention of aflatoxin in the soil. This sorption isotherm is characterized by H-type of Giles classification. When sandy soil was amended with manure, urea, or the both fertilizers, the amount of aflatoxin sorbed on sandy soil significantly decreased as expressed by decreasing Freundlich k_d value which decreased from 774.46 to 9.00, 398.38, and 2.01, respectively. The results of sorption experiments showed that clayey soil rich in clay fraction can detoxificate this toxic compound in soil environmental system due to the high affinity between aflatoxin and clays. Greenhouse experiment showed undetected limit of aflatoxins in plant tissues of tomato (*Lycopersicon esculentum*) and watercress (*Eruca sativa*) cultivated in the two studied soils.

INTRODUCTION

Mycotoxins are toxic compounds that are produced by many species of the Aspergillus, Fusarium, Penicillium, Claviceps, and Alternaria genera of fungi (Huwig et al., 2001). The toxins are secondary metabolites produced by the fungi after infesting grain and other food crops. Biological scientists and agronomists have published numerous studies on mycotoxins research to alleviate these natural toxins in animal feeds and in foods that cause cancer (uncontrolled cellular growth). Yet, aflatoxin persists as a threat to the health of animals and humans in spite of extensive research on preventative measures (Dixon et al., 2008).

Mycotoxins produced or deposited in the soil environment may affect all three aspects of soil quality (Doran et al. 1994), i.e. biological productivity, environmental function and plant/animal/human health. These mycotoxins can enter the soil environment through animal urine and faeces, Animal manure, seed, growing plants and plant debris, seepage from ensiled forage crops, discarded crops, and waste kernels. The fate of mycotoxins formed in situ or added to the soil will depend on interactions with climatic conditions, soil textural and structural characteristics, water fluxes, plant growth and the activity and composition of the soil biota (Elmholt, 2008). Experimental evidence is extremely sparse and mostly originates from laboratory experiments. Mycotoxins may be immobilised in soil by adsorption to soil particles. Some aluminosilicate-containing clays adsorb several mycotoxins, including aflatoxins, Zearalenon (ZEA) and Ocratoxin (OTA). This is used as a means of detoxifying animal feed (Huwig et al. 2001). The ability of clay silicate minerals to adsorb, e.g., ZEA increases with increasing hydrophobicity of the clay (Lemke et al. 1998). Using leachate columns, Madden and Stahr (1993) found no Aflatoxin B1 (FB1) in eluates or soil extracts and concluded that AFB1 was either irreversibly bound to the silty clay loam or chemically altered. Aflatoxin is also strongly adsorbed to soil, especially to loamy soils with high contents of montmorillonite (Angle and Wagner, 1980; Mertz et al., 1981). Goldberg and Angle (1985) established the adsorption coefficients of AFB1 in four different soils and found it to be highest in silty clay loam (238 mg kg^{-1}) and lowest in sandy loam (17 mg kg^{-1}). Moreover, Williams et al. (2003) concluded that FB1 from maize debris in field conditions might enter the ground water under certain environmental conditions.

It is likely that mycotoxins will be subjected to microbial degradation in the soil environment but available results are very sparse. Angle and Wagner (1980) showed that AFB1 added in methanol to a silty loam soil was reduced to the much less toxic AFB2 and AFG2 within a few days, indicating the process to be chemical. Microbial decomposition of C^{14}-AFB1 was measured as $C^{14}O_2$, 14% was degraded after 112 days. Mortensen et al. (2006) found that both OTA and ZEA

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were degraded very fast. The degradation data were fitted well by the sum of two first-order reactions, representing an initial very fast degradation and a second, slower transformation.

In the current study we examined the ability of two different soils to retain aflatoxins (B1, B2, G1, G2) through sorption experiments in batch system. The main objectives of this study, therefore, were: (1) to investigate the sorption behavior of aflatoxins in two soils differed in physicochemical properties, (2) to evaluate the effect of urea and poultry manure fertilizers on the sorption behavior of the aflatoxins, (3) to assess the role of soil microorganisms on aflatoxins degradation through sorption experiments studied time, and (4) to investigate the ability of plants for aflatoxins uptake from those soils (un)treated with or/both fertilizers.

MATERIALS AND METHODS

Physicochemical characterization of soils

Two soils with different properties (clayey and sandy) were selected for the study and collected (0-15 cm depth) from two different locations. The clayey soil (Typic Torrifluvents) was collected from Tanta region while the sandy soil (Typic Quart zipasmments) was collected from Elbostan region 80 Km south west Alexandria city. Sub-samples of the air-dried soils were ground to pass a 2-mm sieve prior to the following chemical analysis: pH in 1:2 soil to water ratio; electrical conductivity (EC) and soluble cations and anions in method soil-water paste extract (Richard, 1954); organic matter by dichromate oxidation (Nelson and Sommers, 1982); cation exchange capacity (CEC) by IM NaOAC (Rhoades, 1982); particle size distribution by the hydrometer method (Day, 1965); total carbonate by means of a calcimeter (Nelson, 1982); water holding capacity (WHC) was determined according to Skene, et al.,(1995). The data of some selected properties of the two soils are summarized in Table 1.

Soil treatments

Organic and mineral fertilizers were applied to the studied soils. Poultry manure was used as a source of the organic fertilizer which obtained from poultry farm of Regional Center for Food and Feed. Urea fertilizer was utilized as a mineral fertilizer.

Sterilization of soils

In order to study the effect of soil microbial activity on aflatoxins degradation, sub-samples of the two soils were sterilized using sodium azide (Skipper & Westermann, 1973). This method can be explained as follow: Samples of soil (25 g dry weight) were placed in 125-ml Erlenmeyer flasks, moistened to field capacity by distilled water, and allowed to equilibrate for 2 days. The samples were then treated with sodium azide, NaN_3 (400 or 800 parts/10^6 on a soil basis, in 1 or 2 ml of water). The flasks were stoppered with cotton plugs and covered with 1 mil polyvinylidene chloride for 2 days at 27 ± 2°C. The film was then removed and the flasks were evacuated for 2 days in a laboratory exhaust hood. Subsamples from treated and untreated soils were removed 2 days after treatment for biological evaluations and sorption experiments.

Aflatoxins preparation

The working solution of aflatoxins was prepared using a mixture of pure aflatoxin standards (AFB_1, AFB_2, AFG_1, and AFG2) at concentration 1 mg/ml for the final mixture. These standards were purchased from Sigma-Aldrich Chemical Company, USA.

Sorption experiments

Sorption experiments were run in batch system to elucidate the sorption behavior of aflatoxins B1, B2, G1, and G2 (as a mixture) by the two studied soils. Also the sorption experiments were run in the presence of the organic fertilizer, urea, or the organic fertilizer with urea to demonstrate the effect of these treatments on the quantity of the aflatoxins retained by the studied soils. Thus, sorption isotherms of the aflatoxins for each soil were obtained by adding 20 ml of the aflatoxins in concentration range of 5 to 160 μg L^{-1} to 3 g soil in a 50-ml centrifuge tube. The tubes were then mechanically shaken on a horizontal shaker (200 rpm) for 12 hours as expected time for equilibration, at room temperature (≈ 25° C). The experiment included triplicate samples and appropriate blanks of each soil and aflatoxins. Samples were centrifuged at 4000 rpm for 15 min, then, 10 ml of the supernatant (equilibrated solution) was transferred to a test tube using a 10 ml pipette. The samples were stored in a freezer for analysis. Aflatoxins concentrations in the collected samples were measured using HPLC. Aflatoxin retained by soils was calculated from the difference between initial amount added and that at equilibrium.

To study the effect of three treatments, mentioned above on the sorption behavior of the aflatoxins, the above sorption experiment was repeated with some modifications. The organic fertilizer or urea was added to the 3 g of each soil at rate of 31.7 or 0.0002 g Kg^{-1} soil, for each treatment, respectively to represent the application rate in the field. Shaking, centrifugation, and analysis were run as previously described in the absence of these treatments.

Table 1. Selected chemical and physical characteristics of the two studied soils

Soil	pH[a]	EC[b] dS m^{-1}	Particles size %			T[c] $CO_3^{=}$ %	OM %	CEC cmol kg^{-1}	WHC[d] g kg^{-1}
			Clay	Silt	Sand				
Clayey	8.29	0.75	44.60	13.50	41.90	3	1.9	33	259.3
Sandy	8.26	3.19	5.00	2.500	92.50	3.6	0.4	6	093.8

[a]pH was measured in 1:2 soil to water ratio
[b]EC was measured in soil water paste extract
T: total.
[d]WHC; water holding capacity

Also, the above sorption experiments were repeated for one concentration (160 ppb) using sterilized soils to demonstrate the role of soil microorganism on aflatoxin degradation within the time equilibration

Greenhouse Experiment:

Two rates of organic or urea fertilizer (0 and 31.7 or 0 and 0.0002 g Kg^{-1} soil, respectively) or the both treatment at the same rate were applied to each soil and thoroughly mixed. Soil for each treatment was transferred to a large plastic bowl. Two- thirds of the water required to obtain field capacity was initially added to the soil with a water dispenser and mixed thoroughly to form a uniform soil-treatment-water mixture. Treated soils mixture was then well mixed with the prepared aflatoxins at two aflatoxins levels (50 and 100 µg kg^{-1} soil). Treated soils mixture was then transferred to a plastic pot (3 kg pot^{-1}) and brought to field capacity, then left for 3 days to reach equilibrium.

Tomato (*Lycopersicon esculentum*) or watercress (*Eruca sativa*) was sown in the prepared pots. Pots were irrigated with tap water to bring the soil moisture to 70 % of field capacity. The plant shoots and roots or fruits were harvested separately after 1 and 3 months for watercress and tomato, respectively and immediately were washed thoroughly with running tap water and rinsed three times with deionized water and then placed on filter papers for air drying. Hereafter, separated plant tissues of different treatments were used for aflatoxins analysis.

Aflatoxins analysis

Aflatoxins were determined using HPLC technique (Agillent 1100 Series U.S.A. with column C_{18} Lichrospher 100 RP-18, 5µm-25cm) according to the following technique. The mobile phase consists of water: methanol: acetonitrile (54:29:17, v/v/v) at flow rate 1 ml/min. and the excitation and emission wavelengths for all aflatoxins were 362 and 460 nm (Florence detector), respectively (Roos et al., 1997).

RESULTS AND DISCUSSIONS

Aflatoxin Sorption Isotherms

Fig. 1 illustrates the sorption isotherm of the aflatoxin by the sandy soil. For describing sorption of aflatoxin in the studied soils, it is convenient to refer to the isotherm classification proposed by Giles et al. (1960) who distinguished four main types of isotherms corresponding to different solute-sorbent interactions. This sorption isotherm for the aflatoxin was of type H of the Giles classification which indicates the high affinity between aflatoxin and sandy soil. The sorption isotherm of the aflatoxin for the clayey soil was not shown due to the undetected aflatoxin concentration in soil-aqueous phase which can be attributed to the high ability of this clayey soil to retain the aflatoxin in soil-solid phase. Many studies demonstrated that clay additions can effectively reduce aflatoxin toxicity to animals (Kiran et al., 1998, Abbès et al., 2008, Juan-juan et al., 2010, White et al., 2013). The mechanism of aflatoxin adsorption by hydrated sodium calcium aluminosilicate (HSCAS) clay can be interpreted according to Phillips et al. (1995) who suggested that adsorption might involve the β-dicarbonyl system of aflatoxin through the chelation of metal ions at the surface and within the interlayers of the HSCAS phyllosilicate clay. Furthermore, the spacings of the aflatoxin-saturated smectites are consistent with a molecule that is parallel with clay layers (Kannewischer et al., 2006). The occupancy of the interlayer surfaces has been calculated to be about 84% based on the size and shape of the molecule and the amount of aflatoxin adsorbed i.e. relatively complete. Thus, the high content of clay fraction in the clayey soil (44.60 %), as shown in Table 1, could be responsible for the very high retention of aflatoxin in this soil. However, the disappearance of aflatoxin from solution can be related to either sorption of aflatoxin by soil- colloidal surface or degradation of the aflatoxin by soil microorganisms. The data obtained from sorption experiment at 160 ppb for sterilized/non-sterilized two studied soils showed that no differences between aflatoxin concentration at equilibrium between sterilized & non-sterilized soils, indicating non

significant effect of biodegradation within the studied equilibrium time and all the aflatoxin disappearance from the soil-aqueous phase is referred to sorption phenomena.

The effect of adding OM, urea, or OM plus urea treatments on aflatoxin sorption isotherm for sandy soils is also shown in Fig.1. The amount of aflatoxin sorbed on sandy soil significantly decreased as a result of adding these treatments with more pronouncing for OM plus urea treatment. Aflatoxin sorption isotherms changed from H-type to S-type as a result of amendment soils with all treatments indicating shifts toward soil-aqueous phase. Again the sorption isotherms for clayey soil as a result of amendment soil with the three treatments were not shown due to the undetected aflatoxin concentration in soil-aqueous phase. This finding insures the role of clay fraction in removing the aflatoxin from the aqueous phase and moving it toward the solid phase even in the presence of organic/urea fertilizers which can contribute in detoxification of this toxic compound in soil environmental system. On contrary, the sandy soil cheap in clay fraction (5%) as seen in Table 1 can easily affected by such organic/urea fertilizers leading to encouraging the presence of aflatoxin in soil-aqueous phase which may cause hazardous impacts on human health via entering food chain or leaching towards ground water.

Sorption Equilibrium Model

The aflatoxin sorption data were plotted according to the Freundlich and Langmuir models. The best fit model for the experimental data was determined according to the coefficient of determination (R^2). The aflatoxin sorption isotherms confirmed better to the Freundlich equation than to the Langmuir equation as indicated by the high values for (R^2). This may be explained by the assumptions inherited in the Langmuir model which may not be valid for a heterogeneous soil system.

The general form of Freundlich model is:

$S=K_dC^n$

Where S is the amount of aflatoxin sorbed by the solid phase (µg/Kg), C is the equilibrium aflatoxin concentration in liquid phase (µg/L), K_d is the distribution coefficient (L/kg), n is the empirical constant describing sorption (non) linearity.

Freundlich model successfully described the aflatoxin sorption over an initial concentration range between 10 and160 µg/L. Freundlich isotherms for the aflatoxin in sandy soil are shown in Fig. 2. Also Freundlich parameters and their R^2 values for the aflatoxin and sandy soil are shown in Table (2). Distribution coefficient (K_d) of sorption isotherms was obtained from the intercept of the linear regression equation and is often used to indicate the capability of a soil to retain a solute and also the extent of its mobility in a liquid phase (Reddy and Dunn, 1986). Low values of K_d indicate that most of the solutes present in the system remain in the solution and hence is available for various chemical processes and plant uptake; while, higher values of K_d reflect a high affinity of solid soil components for solutes. Average K_d value of aflatoxin was 774.46 L/kg for sandy soil (Table 2).

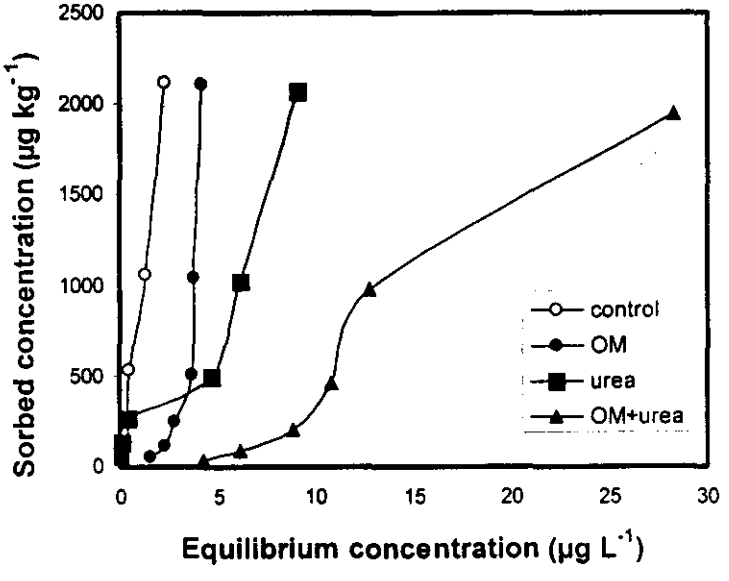

Fig1. Aflatoxin sorption isotherm onto the sandy soil as affected by organic/urea treatments. OM, organic matter amendement

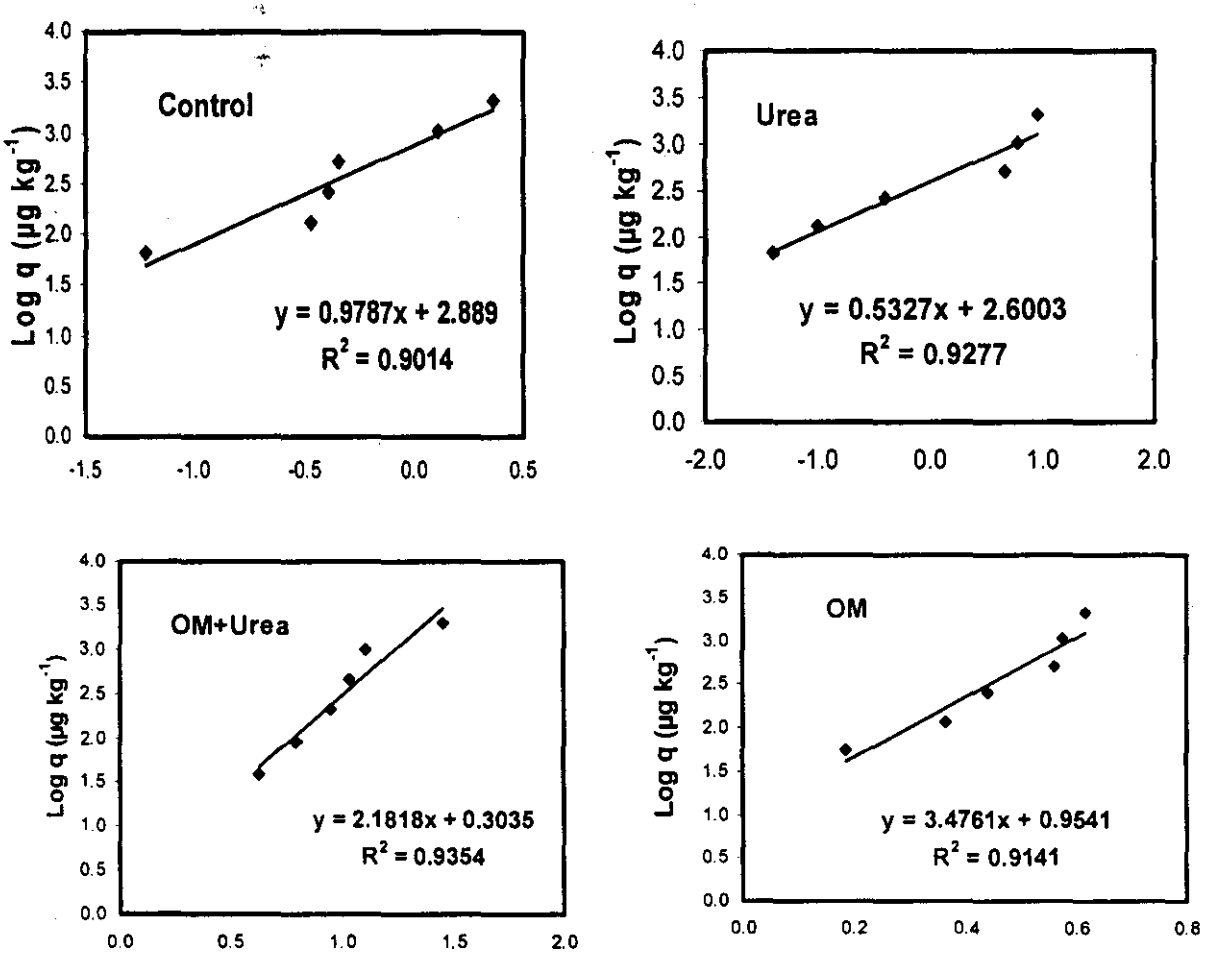

Fig 2. Freundlich sorption isotherm for the aflatoxin onto the sandy soil as affected by organic/urea treatments. OM, organic matter amendement

Due to the lack of literature concerning the sorption isotherm of aflatoxin in soils, this k_d value can be compared with other organic pollutants-K_d in soil of similar properties (Elmholt, 2008). Thus, a review focused on the mobility and leaching of glyphosate herbicide in agriculturally used soils was used for the comparison. In general, glyphosate is considered to be strongly sorbed on the soil such as aflatoxin (Vereecken, 2005). In the current study, the k_d value of the aflatoxin was relatively high comparing with the average of the k_d values of the glycophosate.

The effect of the three fertilizers treatments addition to the sandy soil on aflatoxin sorption can be compared using Freundlich equation coefficient (K_d) - Table 2. The Freundlich K_d value for OM-amended sandy soil dramatically decreased from 774.46 to 9.00 L/kg. It is well known that addition of OM to soil can contribute to increase the cation exchange capacity of soil surface colloid and hence increase the adsorptive capacity of the soil. In the case of the aflatoxin, the ability of the soil to retain this compound decreased as a result of adding OM which may be referred to the low ability of aflatoxin to bind with OM. Blüthgen and Schwertfeger (2000) demonstrated that organic substances tend to be poor adsorbents for aflatoxin. Amendment sandy soil with urea also significantly decreased the K_d value from 774.46 to 398.38 L/kg. Anyway, Soil exchange surface can retain urea in ammonium form (NH^+_4); since this form can compete with aflatoxin for soil-sorption sites causing decrease of aflatoxin sorption. Moreover, Freundlich K_d value for aflatoxin hardly decreased from 774.46 to 2.01 L/kg in sandy soil as a result of adding the both treatments.

Table 2. Freunlich parameters of aflatoxin derived from sorption isotherms for the sandy soil as affected by organic and urea treatments

Treatment	k_d	n	R^2
Control	774.46	0.98	0.90
OM	009.00	3.48	0.91
Urea	398.38	0.53	0.93
OM+Urea	002.01	2.18	0.94

OM, organic matter amendement

Bioavailability experiment

Tomato (*Lycopersicon esculentum*) and watercress (*Eruca sativa*) were chosen in the current study to test the behavior of aflatoxins in soil environment via plant uptake under effect of some agricultural practices such as organic or mineral amendements. The result of this experiment showed undetected limit of aflatoxins in the both harvested plants tissues cultivated in the two studied soils. This finding indicates the low ability of both plants to absorb the aflatoxins even in the more availability conditions in the sandy soil amended with organic or urea fertilizers as discussed in sorption experiments. However, other explanation can be produced as aflatoxins may be absorbed by the studied plants and converted into plant tissues to other derivatives which can not determined with traditional methods. In general, further studies are needed to test such explanation by using other determination methods or bioassay experiments.

CONCLUSIONS

The mobility of aflatoxins in the two studied soils (clayey and soils) was very low, as expressed by Fruendlich K_d values, as a result of high ability of soil colloidal system for aflatoxins sorption. This finding was more pronouncing in clayey soil with high clay content which can contribute in detoxification of aflatoxins in soil environment. When sandy soil was amended with organic/mineral fertilizers, significant increase of aflatoxins mobility, as expressed by increasing Fruendlich K_d values, was noticed. Although increasing aflatoxins availability was noticed in sandy soil as amended with organic/urea fertilizers, undetected limit of aflatoxins in the two plant tissues was shown; however, potential environmental risks can occur due to enhancement aflatoxin presence in soil-aqueous phase under such conditions leading to potential groundwater contamination.

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الملخص العربي

تأثير الاراضى الملوثة معمليا بالأفلاتوكسين على الامتصاص والإتاحة الحيوية له

أمل عبد العزيز ابوحجر، محمد لطفى محرم، محمد عبد المطلع عطوة، خالد مصطفى المليجى

أجريت هذه الدراسة لدراسة سلوك وادمصاص والإتاحة الحيوية لمجموعة أفلاتوكسين (B1, B2, G1, G2) في نوعين من الاراضى (الطينية والرملية) المختلفة في الخواص الكميائيه والفزيائيه وأيضا دراسة هذا السلوك نتيجة معامله هذه الاراضى بسبعض الاسمده العضوية (السماد البلدي) والاسمده المعدنية (اليوريا).

لتحقيق هذا الهدف تم أجراء تجارب الادمصاص فى نظام مغلق مستخدما سلسلة من التركيزات للأفلاتوكسين (٥- ١٦٠ جزء فى البليون).

أيضا تم أجراء تجارب الادمصاص عند تركيز ١٦٠ جزء في البليون على الاراضى تحت الدراسة والتي تم تعقيمها باستخدام ماده Sodium Azide لبيان تأثير فعل ميكروبات التربة على تحطم الأفلاتوكسين خلال فترة تجربة الأدمصاص، كذلك تم إجراء تجربة زراعة في الصوبة لتوضيح تأثير الخواص المختلفة للاراضى المستخدمة على امتصاص نباتات الجرجير و الطماطم للأفلاتوكسين في وجود وغياب المعاملات العضوية أو المعدنية

أوضحت نتائج الدراسة الاتي:

بالنسبه لتجارب الادمصاص، التربة الطينية لم يتواجد تركيزات محسوسة بالنسبة للأفلاتوكسين فى الطور المائي للتربة والتي يمكن أن تعزى إلى قوة ادمصاص هذا المركب على الطور الصلب لهذه التربة وليس إلى تحطم الافلاتوكسين بفعل ميكروبات التربة، حيث استدل من تجربة الادمصاص للاراضى المعقمة انه لا يوجد تأثير واضح لميكروبات التربة على تحطم الافلاتوكسين خلال مدة التجربة.

بالنسبة للتربة الرملية، منحنيات الادمصاص دلت على وجود ادمصاص قوى للافلاتوكسين على تلك التربة. عند معاملة هذه التربة بالسماد البلدي أو اليوريا أدى إلى انخفاض ادمصاص الافلاتوكسين على سطح التبادل لهذه التربه (معبرا عنها بثابت K_d المحسوب من معادلة فريندلخ).

بالنسبه لتجارب الإتاحة الحيوية، لم يتواجد تركيزات محسوسة للأفلاتوكسين في أنسجة النباتات المختلفة

النتائج النهائية اوضحت ان التربه الطينيه الغنيه فى محتواها من معادن الطين يمكن ان تعمل على أزاله سميه الافلاتوكسين في النظام البيئي للتربة نتيجة لقوه الادمصاص ما بين الأفلاتوكسين ومعادن الطين.

على النقيض بالنسبه للتربة الرملية وفى حاله استخدام بعض الاسمده العضوية أوالمعدنية، بعض المخاطر البيئية يمكن أن تحدث نتيجة لتشجيع هذه المعاملات على تواجد الافلاتوكسين فى الطور المائي للتربة مما قد يسبب تلوث الماء الجوفي بالأفلاتوكسين.