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Uptake of Three Pharmaceuticals by Beans (*Phaseolus vulgaris* L.) from Contaminated Soils

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Abstract:

The ability of beans (*Phaseolus vulgaris* L.) to uptake three pharmaceuticals (diclofenac, mefenamic acid and metronidazole) from two types of soil (clay and sandy soil) was investigated in this study to explore the human exposure to these pharmaceuticals via the consumption of beans. A pot experiment was conducted with beans plants which were grown in two types of soil for six weeks under controlled conditions. During the experiment period, the soil pore water was collected weekly and the concentrations of the test compounds in soil pore water as well as in plant organs (roots, stems and leaves) were weekly determined. The results showed that the studied pharmaceuticals were detected in all plant tissues; their concentrations in plant roots were higher than plant stems and leaves. The extent level and accumulation of the studied pharmaceuticals in sandy soil was higher than the clay soil. The concentration of diclofenac in plant tissues was higher than both of mefenamic acid and metronidazole, indicating that diclofenac is more available to plant. The content of dissolved pharmaceuticals in soil pore water decreased gradually over time during the experimental period confirming the ability of beans to uptake these pharmaceuticals from soil. The results suggest the possibility of studying pharmaceuticals to be accumulated in beans tissues despite their low concentrations in the studied soils.

Key words: Bioconcentration factor, Contaminated soil, Pharmaceuticals, *Phaseolus vulgaris* L., Soil pore water.

Introduction:

Over the last decade, pharmaceuticals have been recognized as potential environmental contaminants according to their effects on aquatic and terrestrial organisms (1-3). The main source of the pharmaceuticals in the environment is related to human excretion, uncontrolled and illegal drug disposal and hospital effluents (4-6).

Most studies have demonstrated that pharmaceutical products are not fully metabolized in the body, not completely eliminated from water during the treatment of wastewater and not completely degraded in the environment (7-8). Therefore, the soil environment will be exposed to these chemical products with their metabolites when soil is irrigated with reclaimed wastewater effluent and when sludge is applied to soil as an amendment.

When pharmaceuticals and their bioactive metabolites reach the soil layers, they may either transport to plants or be readily to accumulate in groundwater through leaching and surface runoff (9), which can be found in the environment at concentrations ranging from parts-per-billion to parts-per-million. However, even though these concentrations are low, the development of new pharmaceuticals resulted in their continual input into the environment leading to negative effects on the environment.

Some studies have reported that diclofenac that belong to the group of non-steroidal anti-inflammatory drugs (NSAIDs) can inhibit the synthesis of prostaglandin E₂ in *M. galloprovincialis* at 100µg/L (10). Additional

studies have reported that the exposure to a concentration ranged from ng to µg/L causes an oxidative stress in fish and mussels (11-12). Mefenamic acid which belongs to the anthranilic acid derivatives class of NSAIDs drugs is used for pain treatment. The overdose of mefenamic acid can cause an accumulation of a toxic metabolite which leads to bloody diarrhea, vomiting and nausea (13). Metronidazole is classified as antibacterial agent used in the treatment of various anaerobic infections. Some studies have demonstrated that metronidazole causes a toxic effect in the tissues of the intestines of the fish (*Onchorhynchus mykiss*) (14).

A number of studies have demonstrated that in soil, and depending on the physicochemical properties of the pharmaceuticals and soil characteristics (e.g., pH and organic matter content), the pharmaceuticals might be retained by soil or mobilized by percolating water (15). On the other hand, highly mobile pharmaceuticals have the potential to be taken up and accumulated into plant species including roots and shoots of plants (16-18).

Previous studies have focused on evaluating phytotoxicity or plant uptake of pharmaceuticals which could enter terrestrial food chain through soil by the application of biosolids and wastewater or associated with recycled manure produced from animal wastes (19). Recently, uptake of human pharmaceuticals by plants grown hydroponically or in nutrient solution has also been reported for assessing phytotoxicity effects on different crop plants (20-21).

From literature, a number of publications have presented the detection of a range of pharmaceuticals in the soil as well as their uptake by various types of plants, but very few studies have

explored the use of bean plants (e.g. pinto bean "*Phaseolus vulgaris* L.") to uptake the pharmaceuticals from contaminated soils or the use of Rhizon soil moisture samplers to assess the pharmaceuticals available concentration in soil pore water (22-24).

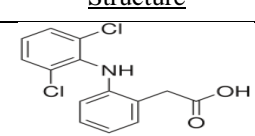
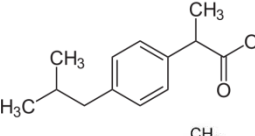
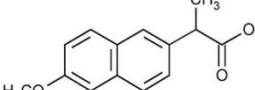
The current work aims to demonstrate: *i*) the ability of beans "*Phaseolus vulgaris* L." to uptake three pharmaceuticals including diclofenac (DCL), mefenamic acid (MEF) and metronidazole (MET) into different parts of beans (roots, stems and leaves) grown in soil exposed to these pharmaceuticals in greenhouse pot experiment. *ii*) the temporal changes in the soil pore water pharmaceutical concentrations during the experiment period, and *iii*) provide an experimental data for improvement of the plant uptake and to explain the possible risk of pharmaceuticals to terrestrial organisms via their accumulation into the food chain.

Materials and Methods:

Chemicals and reagents

Analytical standards of three pharmaceuticals (Table 1), DCL (>98% purity), MEF (>99% purity) MET (99.9% purity) were kindly donated by a local pharmaceutical industries and were used as reference standards without further purification. HPLC grade acetonitrile was obtained from Merck. Ultrapure water (>18 MΩ) was prepared by "Thermo Scientific Barnstead Easy pure II systems". The stock solutions were prepared from the tested pharmaceutical standards in acetonitrile. Working standard solutions were prepared and diluted from stock standard solutions.

Table 1. Molecular structures and main physical–chemical properties of the studied pharmaceuticals.

Compound	M.W	log Kow ^a	pKa ^b	Structure
Diclofenac	296.14	4.51	4.15	
Mefenamic acid	206.29	3.97	4.91	
Metronidazole	230.25	3.18	4.15	

^aLogarithm of the octanol–water partition coefficient.

^bAcidic ionization constant.

Soil sampling

Two types of uncontaminated locally garden soil (clay and sandy soil) with different properties (Table 2) were used in this study. The selected soils were obtained from the top 0-20 cm depth with a stainless steel spade, carefully bagged in clean polyethylene bags before transporting to the laboratory. Prior to testing, the soils were air-

dried and sieved to 2mm to ensure homogeneity'. The physicochemical properties of the soils were determined in laboratory according to standardized French (AFNOR 1999) or international (ISO 1999) procedures. The selected soils were not previously cultivated and did not receive biosolids or wastewater applications.'

Table 2. Physico-chemical properties of the tested soils

	pH	OM%	CaCO ₃ %	Sand%	Silt%	Clay%
Clay soil	7.73	8.3 ± 0.11	33 ± 2.3	28	20	52
Sand soil	7.41	3.1 ± 0.21	12.7 ± 1.7	76	9	15

OM: organic matter

Germination test

A pot experiment was conducted with the selected soils for use in bean plant uptake study. For each pharmaceutical, pots were prepared in five replicates. Bean seeds were soaked for 4h prior to planting, to aid in germination, then, it transferred into petri dishes on moist cotton for germination at 25°C.

Approximately 500 ± 5g of soil was potted in plastic plant pot. For each pot, two seedlings of dwarf bean were transferred, and later thinned to one after emergence. The soil in each pot series was spiked one time a week with an aliquots of 1g/L (in acetonitrile) solution to give a concentration of 5µg/g of the test compounds diclofenac, mefenamic acid and metronidazole separately. This concentration was chosen in order to detect the tested pharmaceuticals in soil pore water.

In a growth chamber, bean plants were left to grow for 6 weeks in controlled conditions (16h light/8h darkness, 25°C/20°C) with 55 - 65% humidity. A control soil with plants without pharmaceuticals addition were included to examine the ability of plants uptake, watered daily with Milli-Q water. Control soil with pharmaceuticals and no beans were also included to assess the possible degradation of pharmaceuticals in the test soils."

During the experiment period, the bean plants were watered once a week with a nutrient solution (200 mg/L) of Ca(NO₃)₂, KNO₃, MgSO₄, KH₂PO₄, ZnSO₄, and NH₄NO₃

Plants and soil analysis

After six weeks of growth, plants were harvested, rinsed with deionized water, and separated into roots, stems and leaves. Washed plant samples were dried at 80°C for 72h before recording the dry matter yield, then ground with a laboratory grinder.

Plant samples were extracted with 10ml of acetonitrile: water 1:1(v/v) in test tube. The test

tube was vortexed for 2min, ultrasonicated for 15min. The samples were centrifuged for 25min at 2000 rpm, and the supernatant was removed. The sample solutions were then extracted by solid phase extraction with an Oasis HLB cartridge and eluted with acetonitrile. The eluate was evaporated to near dryness and finally diluted with acetonitrile:water up to 1ml for HPLC analysis.

As for soil analysis, in 50ml of screw-top Teflon centrifuge tube, 25ml of acetonitrile was added to 1g of dried test soil, ultrasonicated for 15min, centrifuged at 3000rpm for 15min, and decanted the supernatant. The supernatant was filtered through a 0.45µm PTFE syringe filter, and then analyzed using HPLC to determine the amount of test pharmaceuticals remaining.

Soil pore water (SPW) was collected every week during the cultivation period using Rhizon soil moisture samplers "Rhizosphere Research Products, Wageningen, The Netherlands". Distilled water was added to the soils to maintain 80% of the WHC (water holding capacity). The system was allowed to equilibrate for 24hr before SPW collections."

Analytical Conditions of HPLC

HPLC "High Performance Liquid Chromatograph" system with UV-VIS detector was used for the determination of diclofenac, mefenamic acid and metronidazole. The analysis of test pharmaceuticals was performed on a stainless steel C18 column (5µ, 150 mm × 4.6 mm: Shimadzu, Japan). The mobile phase was composed of acetonitrile: water (80:20, v/v), with the pH value adjusted to 2 with acetic acid. Flow rate was kept at 0.8 mL min⁻¹, and the eluent was monitored at 280nm, 285nm and 289nm for diclofenac, mefenamic acid and metronidazole respectively.

Bioconcentration factor (BCF)

The BCF "the ratio of the chemical compound concentration in bean plant to its

concentration in soil" can be calculated from the equation (24):

$$BCF = \frac{\text{Concentration of test pharmaceuticals in plant organ}}{\text{Concentration of test pharmaceuticals in dry soil}}$$

Statistical analysis

The results of the test pharmaceutical concentrations in bean plants as well as in soil pore water were analyzed with statistical software package (SPSS, Chicago, IL, USA). Analysis of variance (ANOVA) was used for the statistical comparisons of the resulted data. Statistical tests were considered significant at $P \leq 0.05$.

Results and Discussion:

Uptake of test pharmaceuticals by plant

The concentration of the tested pharmaceuticals (DCL, MEF and MET) in different parts of beans (roots, stems and leaves) during the experimental period (42 days) is shown in Fig.1, 2 and 3.

These Figures show that test pharmaceuticals were taken up in detectable quantities by bean plants. In the control plants, none of the study compounds was detected. Also, in the control soil without plant, the level of the tested compounds was stilled constant with negligible decrease in their concentration, indicates that the pharmaceuticals photodegradation did not perhaps occur.

DCL was detected in all plant organs in both clay and sandy soils at levels ranging from 11.5 – 22 mgkg⁻¹, 8.9 – 15 mgkg⁻¹, and 4.6 – 12 mgkg⁻¹ in roots, stems and leaves dry weight respectively.

MEF was detected at levels ranging from 5.3 – 7.6 mgkg⁻¹, 2.9 – 4.3 mgkg⁻¹, and 2.5 – 3.3 mgkg⁻¹ in roots, stems and leaves dry weight respectively in both types of soils.

Similarly, MET was also detected at levels 7.9 – 12.8mgkg⁻¹, 4 – 8.3mgkg⁻¹, and 3.2 – 6.5mgkg⁻¹ in roots, stems and leaves dry weight respectively.

From Fig. 1, 2 and 3, it can be seen that the concentration of the tested compounds increased over time in plant organs in sandy soil in comparison to clay soil. The accumulation of these compounds in plant roots was higher than plant stems and leaves in both clay and sandy soil.

Also, Figs.1, 2, 3 show that for clay treatment, the concentration of the studied compounds exhibited a little increase in the first two weeks, then the concentration was significantly different (increased $P < 0.05$ and 0.01) during the experiment period.

In sandy soil treatment, there were differences in the concentration of the studied

compounds each week ($P < 0.05$, 0.01 and 0.001). The concentration of DCL in plant organs increased after the first week of planting, the concentration of MEF started increasing after the second week whilst, the increasing of MET concentration started from the third week. The exception was MEF in leaves, the increasing of concentration started from the fourth week.

Among the test compounds detected each week in both clay and sandy soil, the concentration of DCL in plant organs (roots, stems and leaves) was significantly higher than both MET and MEF and the concentration followed the order DCL > MET > MEF, suggesting that DCL was easier to be absorbed by beans tissues than MET and MEF.

The results obtained were in agreement with recent studies. Karnjanapiboonwong (24) reported that the concentrations of the EE2 and triclosan were higher in sandy soils compared to clay soil which might be attributed to organic carbon content in each type of soil. Also, the difference in sorption ability of chemicals between clay and sandy soil will affect the available amount of pharmaceuticals in soil pore water, and thus leading to reduce the amount of pharmaceuticals in plant organs (25).

For all tested compounds, the amounts of these compounds detected after two or three weeks of experiment increased in comparison to the amounts detected only after the first week, which indicates that tested pharmaceuticals are taken up by the plants roots and subsequently translocate them to the plant leaves.

However, the tested compounds were accumulated in roots more than aboveground tissues. This result is in agreement with previous researches, Zhai (26) reported that DCL is found in the roots of *Cyperus alternifolius* at a higher concentration than plant leaves, Dodgen (27) showed that DCL accumulation into lettuce and collards roots was much higher than stems and leaves, and its level in plant organs was higher than the other studied pharmaceuticals. This behavior was similar to other types of pharmaceuticals under hydroponic conditions, Herklotz (20) reported that the concentration of carbamazepin, salbutamol, sulfamethoxazole in cabbage were higher in plant roots than plant shoots suggesting that the roots might be consider as a sink for many pharmaceuticals.

Depending on recent studies, the high level of many of pharmaceuticals in plant roots than plant leaves might be attributed to several factors, such as the concentration of pharmaceuticals in soil solution, pharmaceutical octanol-water partition coefficient, lipophilicity and physico-chemical

characteristics of both pharmaceutical and soil (28-30). However, the lipophilic compounds could be taken up by plant roots through active and passive transport in high level in comparison to plant leaves. The high percentage of lipid content in roots than the other plant organs make pharmaceuticals be preferentially accumulated in plant roots, then translocate to plant aerial parts by transpirational water. Therefore, these properties controlled the uptake of each of DCL, MEF and MET by bean plants and showed a differences in accumulation concentration of these compounds in both clay and sandy soil.

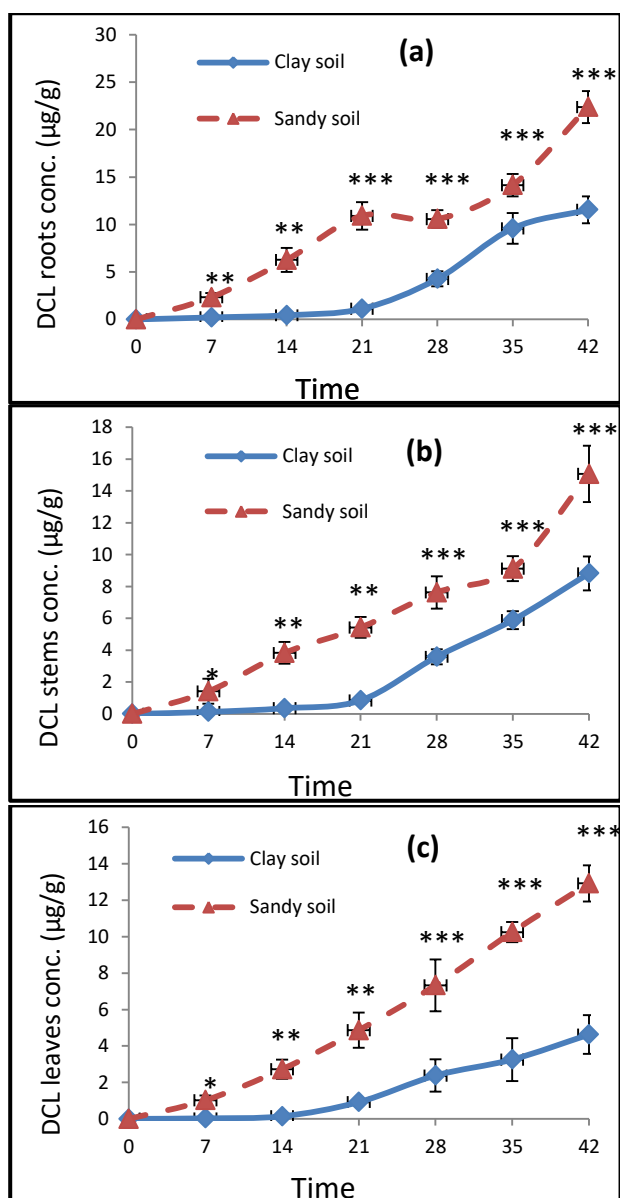


Figure 1. The mean concentration of diclofenac in beans tissues: roots (a), stems (b), and leaves (c) grown in clay and sand soil during 6 weeks. Error bars refer to standard error (n=5). * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ significantly different values between clay and sand soil.

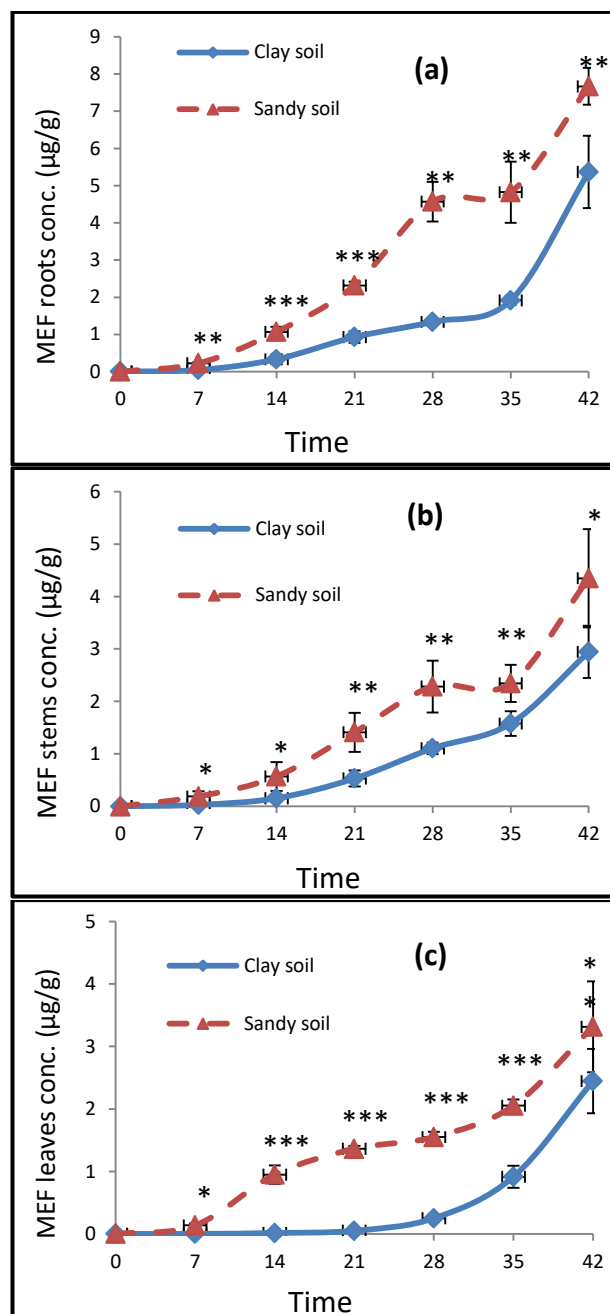


Figure 2. The mean concentration of Mefenamic acid in beans tissues: roots (a), stems (b), and leaves (c) grown in clay and sand soil during 6 weeks. Error bars refer to standard error (n=5). * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ significantly different values between clay and sand soil.

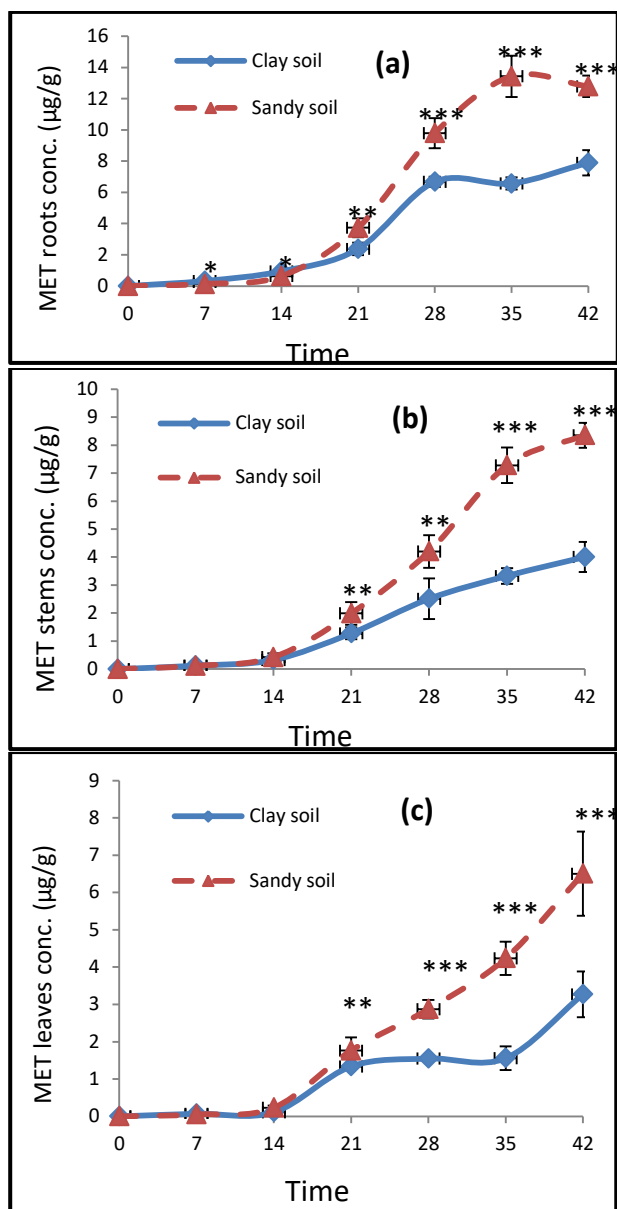


Figure 3. The mean concentration of metronidazole in beans tissues: roots (a), stems (b), and leaves (c) grown in clay and sand soil during 6 weeks. Error bars refer to standard error (n=5). * $P \leq 0.05$, ** $P \leq 0.01$, *** $P \leq 0.001$ significantly different values between clay and sand soil.

Bioconcentration factor

Table 3 presents the bioconcentration factor (BCF) which can be considered as an indicator of the bean plants accumulation behavior. BCF represents the pharmaceuticals translocation from soil to plant organs and can be expressed as the ratio

of the pharmaceutical concentration in plant organs divided by its concentration in soil.

From Table 3, all the studied pharmaceuticals in both types of soil, the BCF has the maximum value in the bean plant roots in comparison to bean plant leaves during the experiment period. Also, the BCF value for the tested pharmaceuticals in beans grown in sandy soil is higher than beans planted in clay soil over time of experiment. In addition, the BCF value for the studied compounds increased gradually with time and reached up to 7.9 for DCL in roots and 4.4 in leaves for plants grown in sandy soil at the end of experiment followed by MET and MEF, whereas, in clay soil, no significant differences in BCF value for the tested compounds were observed. In fact, these values were still less than the work of Karnjanapiboonwong (24) which found greater BCF values in roots and leaves of the pinto bean "*Phaseolus vulgaris*" in both clay and sandy soils for EE2 and triclosan. This can be explained by the potential of tested compounds to accumulate in plant roots, suggesting that the accumulation of DCL was relatively high in bean plant roots and leaves in comparison to test compounds.

The higher value of BCF is related to the fact of the higher pharmaceutical accumulation in plants and exhibited risk of exposure to humans and animals. However, in this study, the MEF and MET had lower risk of exposure to humans and animals in comparison to DCL according to their lower BCF value as compared to DCL.

It is well known that one of the plant roots functions is the absorption of the dissolved components (e.g. pharmaceuticals, minerals ...etc.) that exist in soil pore water and feeding the aboveground tissues. The transport of these dissolved compounds depends mainly on the type of pharmaceutical, its ability of adsorption on soil clay surface, in addition to biological role of the tested plant (31-33). On the other hand, the translocation of the pharmaceuticals towards plant shoots might be attributed to the $\log K_{ow}$ for the tested pharmaceutical (34,35).

In this study, bioconcentration values can be used to estimate the potential risk of the tested compounds to bean plants grown in contaminated soils irrigated with waste water.

Table 3. Bioconcentration factor for test compounds in plants based on dry weight.

Compound	Soil type	Plant organ	Day					
			7	14	21	28	35	42
Diclofenac	Clay	Roots	1.19	1.34	1.79	1.38	1.5	2.01
		Leaves	0.17	0.43	1.47	1.1	0.95	0.78
	Sand	Roots	2.91	3.01	3.51	6.7	6.68	7.94
		Leaves	1.02	1.65	2.03	2.24	2.41	2.81
Mefenamic	Clay	Roots	0.19	0.79	1.06	1.01	1.17	1.93
		Leaves	0	0.03	0.05	0.18	0.56	0.98
	Sand	Roots	0.62	2.32	2.07	2.64	2	2.99
		Leaves	0.37	2.06	1.22	0.89	0.85	0.98
Metronidazole	Clay	Roots	0.77	1.13	1.93	3.85	2.97	3.09
		Leaves	0.16	0.13	1.09	0.89	0.7	0.86
	Sand	Roots	0.21	0.67	2.6	4.81	5.08	4.15
		Leaves	0.08	0.24	1.23	1.41	1.6	1.56

Pharmaceuticals concentration in soil pore water

The dissolved pharmaceuticals concentration in soil pore water for both studied types of soil during the experiment period are shown in Fig. 4.

A significant difference ($P < 0.01$ and 0.001) was observed for DCL concentration in soil pore water between clay soil and sandy soil during the growing period. Fig. 4a showed that the DCL dissolved concentration in sandy soil dropped markedly from the first week to last week of experiment. While, in clay soil, DCL dissolved concentration decreased in the first and second weeks, and then its concentration remained constant towards the end of the experiment.

Similarly to DCL, there was a significant difference ($P < 0.01$ and 0.001) in the concentration of MEF in soil pore water between the studied types of soil (Fig. 4b). The concentration of MEF in soil pore water of sandy soil also decreased gradually through the experiment period from the first week to last week, whereas in clay soil, MEF concentration decreased clearly in the first weeks with little increasing in the middle of experiment and re-decreased in the last week of the experiment.

In the case of MET, the same pattern of difference was also observed in the soil pore water concentration between the studied types of soil ($P < 0.01$ and 0.001) (Fig. 4c).

In sandy soil, the MET concentration in soil pore water decreased over time of the experiment, with a little difference in its level in the last two weeks. The same pattern was also observed in clay soil.

From Fig. 4, it is obvious that the tested compounds in soil pore water were not completely removed by bean plant during the experimental period, while the reductions in their concentrations were at different rates. This result concerning the removal ability of bean plants towards the tested pharmaceuticals can partly be explained by the conditions of the experiment such as the biomass of cultivated plants, biotic processes as well as the abiotic processes of tested pharmaceuticals (36).

Our results showed that the concentration of the studied compounds in soil pore water decreased over time during the experiment period, while their concentration in plant tissues increased, which provides further evidence that the studied soil (especially sandy soil) will not fully retain the sorptive pharmaceuticals making them more available for bean uptake as compared to clay soil. This result is in agreement with the work of Karnjanapiboonwong (24). In addition, the greatest the concentration of the tested pharmaceuticals in soil pore water for sandy soil in comparison to clay soil may have played an important role in their higher concentration uptake by bean plant (Fig 4) (35).

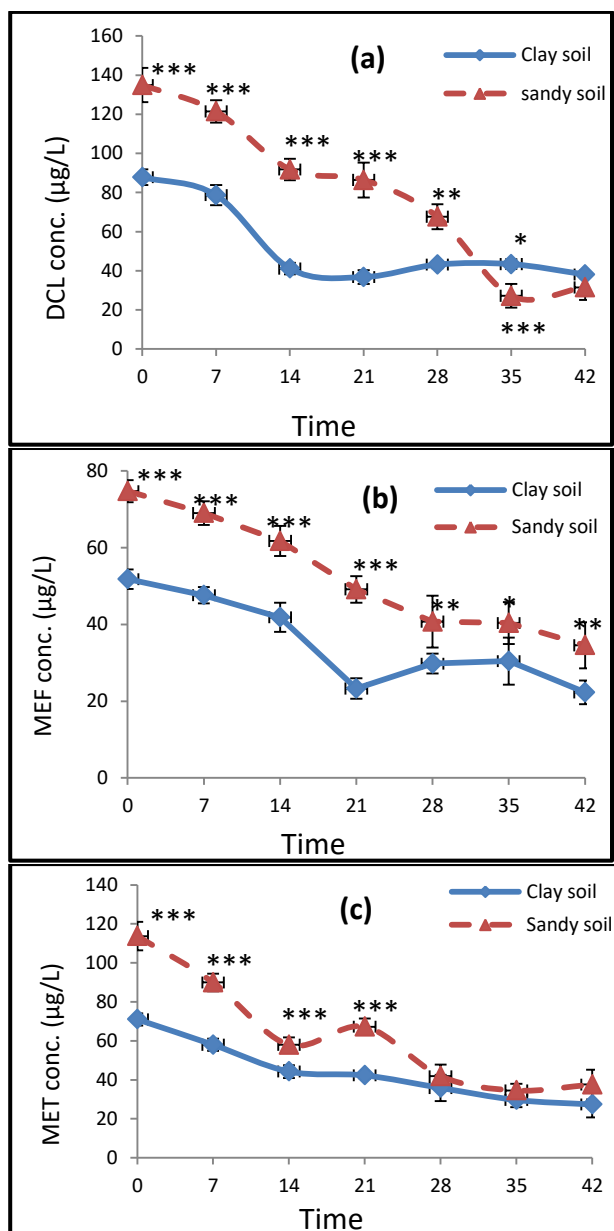


Figure 4. The mean concentration of diclofenac (a), mefenamic acid (b) and metronidazole (c) in soil pore water of beans grown in clay and sand soil during 6 weeks. Error bars refer to standard error (n=5). * $P \leq 0.05$, ** $P \leq 0.01$, * $P \leq 0.001$ significantly different values between clay and sand soil.**

Conclusions:

In our study, the uptake and translocation of three pharmaceuticals from two types of soils into bean plants as an exposure route of these pharmaceuticals to living organisms including humans and animals are investigated.

The studied compounds are found to be taken up by plant roots and accumulate them in their stems and leaves. The concentrations of studied pharmaceuticals in plant roots are higher than stems and leaves and their concentrations in sandy soil are higher than that of clay soil.

Among the studied pharmaceuticals, diclofenac is taken up more than the other compounds by plant organs indicates that diclofenac is more available compared to other compounds.

The dissolved concentration of the studied pharmaceuticals in soil pore water decreases gradually, while their concentration increases in plant organs during the experimental period which supports the ability of beans to uptake the studied pharmaceuticals from soil solution.

It can be concluded that the presence of pharmaceuticals in bean tissues used as feed sources may pose health risks for human and animals when irrigated with contaminated water or in biosolid land application. However, further work is still necessary to understand and explore the mechanisms and the ability of beans to uptake the pharmaceuticals from municipal soils to plant organs.

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Authors' declaration:

- Conflicts of Interest: None.
- We hereby confirm that all the Figures and Tables in the manuscript are mine ours. Besides, the Figures and images, which are not mine ours, have been given the permission for re-publication attached with the manuscript.
- Ethical Clearance: The project was approved by the local ethical committee in University of Technology.

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امتصاص ثلاثة أنواع من الادوية بواسطة نبات الفاصولياء في التربة الملوثة

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الخلاصة:

تم في هذا البحث دراسة قابلية نبات الفاصولياء على امتصاص ثلاثة أنواع من الادوية وهي كل من الدايكلوفيناك، الميفينامك اسيد و الميترونيدازول في نوعين من التربة الملوثة وذلك لاستكشاف التعرض البشري لهذه الادوية عن طريق استهلاك الفاصولياء. تم اجراء التجربة من خلال زراعة نبات الفاصولياء في اوعية بلاستيكية باستخدام نوعين من التربة ولمدة ستة اسابيع داخل المختبر وتحت ظروف زراعية مسيطر عليها. تم جمع محلول التربة اسبوعيا طيلة فترة اجراء التجربة، وتم قياس تركيز الادوية الموجودة في محلول التربة وكذلك تركيزها في اجزاء النبات المختلفة (الجزور، السيقان والاوراق) اسبوعيا ايضا. أظهرت النتائج وجود الادوية قيد الدراسة في كل اجزاء النبات، وكان تركيز هذه الادوية في الجذور أعلى مما هو عليه في كل من السيقان والاوراق. كما ان تركيز الادوية في التربة الرملية أعلى مقارنة مع التربة الطينية. وجد بأن تركيز الدايكلوفيناك في اجزاء النبات المختلفة هو أعلى مقارنة بالميفينامك اسيد والميترونيدازول مما يشير إلى أن الديكلوفيناك هو متاح للنبات بكمية أكثر مقارنة مع الدوائين الاخرين. كما وجد بأن تركيز الادوية المذابة في محلول التربة قد تناقص تدريجيا مع الزمن خلال فترة اجراء التجربة مما يشير الى قابلية الفاصولياء على امتصاص هذه الادوية الموجودة في التربة. أشارت النتائج الى امكانية تراكم الادوية المذكورة انفا في اجزاء نبات الفاصولياء على الرغم من تراكيزها الواطنة في التربة الملوثة.

الكلمات المفتاحية: التربة الملوثة، الادوية، عامل التركيز البيولوجي، نبات الفاصولياء، محلول التربة.