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PHYTOREMEDIATION OF SOIL AND WATER CONTAMINATED WITH DIAZINON

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ABSTRACT

Diazinon is one of the most widely used organophosphate insecticides against different pests. Foliar application of diazinon may lead to soil contamination and washing it's residues to surface water and then enter to the ground water. Phytoremediation is one of the most promising methods to prevent the hazard of such contamination. The present work was aimed to study the potential of two plants in removal diazinon residues from soil and water. *Plantago major* L. and *Helianthus annus* L. plants were tested to clean soil, and *P. major* plant was tested to clean water under laboratory conditions. The results showed that the accumulation of diazinon residues in *P. major* and *H. annus* roots was higher than those of leaves. In soil experiment, diazinon uptaked in the roots of *P. major* to reach the maximum levels after one day (14.77 µg /g). Afterwards, the concentration decreased gradually throughout the test. While, in *H. annus* roots it reached the maximum after 10 days of exposure (16.40 µg/g), then decreased until the end of testing. *P. major* roots were an efficient accumulator of diazinon in short time while *H. annus* roots were an efficient accumulator of diazinon in long time. Diazinon was translocated into *P. major* leaves much faster than *H. annus* leaves. In water, diazinon accumulated in the roots of *P. major* to reach the maximum levels after 1 day (37.80 µg/g), while translocated into the leaves to reach the maximum levels through 7 days of exposure (10.51 µg/g). The obtained results indicated that *P. major* removes efficiently diazinon residues in water, soil and has a potential activity for pesticides phytoremediation.

Key words: Phytoremediation, *P. major*, *H. annus*, diazinon, soil, water.

INTRODUCTION

The contamination of soil and water by pesticides is currently a significant concern throughout the world because many of these compounds are detrimental to both human health and the environment. Among newly developed pesticides, organophosphate pesticides are most commonly used (Memon *et al.*, 2008). This is due to their reduced persistence and shorter half-life as compared to organochlorine pesticides whose use is banned in many countries. Diazinon, (O,O- diethyl- O - 2- isopropyl – 6 – methyl - pyrimidine - 4 -yl) phosphorothioate, is the common name of an organophosphorus pesticide used to control insect pests in soil, ornamental plants, fruit and vegetable as well field crops. It was formerly used as the active ingredient in household and garden products to control pests such as flies, fleas, and cockroaches. Diazinon is a synthetic chemical, it does not occur naturally in the environment (ATSDR, 2006). It is also used throughout the world to control public health, and is applied to control ectoparasites in veterinary medicine (Watterson, 1999). Diazinon is relatively water soluble (40 mg/l at 25°C, non- polar, moderately mobile and persistent in soil; hence, it is of concern for groundwater and surface derived drinking water (Kidd and James, 1991). After its application on crops and plants, diazinon is easily washed into

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surface water and enters the ground water. Eventually, it enters the aquatic environment in large quantities (Dutta and Meijer, 2003; Shemer and Linden, 2006). Because of its aquatic distribution, diazinon affects a wide range of nontarget organisms, like invertebrates, mammals, birds, and fish, especially those inhabiting aquatic environment (Eisler, 1986; Hamm and Hinton, 2000). The half-lives ($T_{1/2}$) of diazinon were found to be 1-5 weeks in nonsterile soil and 6-12 weeks in sterile ones (Howard, 1991). Diazinon applied to soil can be absorbed by plant roots and translocated in plants. Half-lives range from 2 to 14 days. Low temperature and high oil content increase the persistence of diazinon in plants (Kamrin, 1997). Phytoremediation is a strategy that use plant to degrade, stabilize and remove contaminants from soil, water and wastes (Yu and Gu, 2008; Kuo *et al.*, 2014). Phytoremediation is an environmentally sound technology for pollution prevention, control and remediation. There have been several studies focused on the phytoremediation of pesticides (Khan *et al.*, 2011; Romeh and Hendawy, 2013; Iba´n˜ez *et al.*, 2014; Romeh, 2014; Zhang *et al.*, 2014). Sunflower (*Helianthus annus* L.) is a relatively high-biomass and fast-growing accumulator plant which has the ability to take up and accumulate metals and radionuclides (Tassi *et al.*, 2008; Tahmasbian and Sinegani, 2014). The broadleaved plantain (*Plantago major* L.) is a perennial weed and commonly found by roadsides, in meadow land, cultivated fields, waste areas and water canals. The seed and husks of this plant contain high level of fiber which expand and become highly gelatinous when soaked in water (Sharifa *et al.*, 2008).

The aim of this study is to evaluation the phytoremediation potential of *H. annus* and *P. major* for diazinon contaminated soil and water.

MATERIALS AND METHODS

Pesticide and Tested Plants

Diazinon (60% EC), [O, O- diethyl- O - (2-isopropy – 6 – methyl – pyrimidine - 4 -yl) phosphorothioate] was obtained from a local manufactory, El-Nasr Company, Abouroash, Egypt. The common broadleaf plantain (*P. major*) was obtained as seedlings in phytoremediation experiment from meadow land in Zagazig University, Zagazig, Sharkia Governorate, Egypt. Sunflower, (*H. annus*) seeds were germinated in bitmos growing medium and kept moist during the initial growth period (1 week), which may vary according to plant variety. After the germination period, only healthy seedlings with uniform size were selected. Plants were then gently removed from growing medium, and the roots were rinsed from any adhering material before transferring to the soil.

Experimental Design

To evaluate the removal of diazinon from the soil, three treatments were performed in this experiment, and each treatment consisted of three replicates: (1) diazinon contaminated soil without plants, (2) diazinon contaminated soil with *P. major* only (each pot contained one seedling of *P. major*), (3) diazinon contaminated soil with *H. annus* seedlings (each pot contained eight seedlings of *H. annus*). A whole-plant uptake experiment was performed on soil in a pot experiment for 21-days exposure. Air-dried sieved clay loam soil (organic matter, 1.79%, pH 7.8, electric conductivity 2.36) was obtained from Kamrona village, Menia El-Kamh district, Sharkia Governorate, Egypt, and then placed in plastic pots. The pots were filled with 500 g of air dried soil. After planting, diazinon dissolved in water was spiked into the 150 ml of distilled water used for irrigation to obtain original concentration of 20 µg /g. The irrigation water containing diazinon was dropped into the pots with a caution to avoid the direct contact of plant leaves. After 1, 3, 7, 10, 14 and 21 days, exposed and control plants were collected. Plant roots from soil were rinsed in running tap water for 2 min and were blotted dry. The plants were dissected into individual roots, stalks and leaves in the case of *H. annus*, while to roots and leaves in the case of *P. major*; then, 4 g of leaves, roots, stalks each and 20 g of soil were analyzed for the pesticide. All pots were watered with 50 ml tap water every 4 days or additionally watered when necessary. A whole broadleaf plantain was grown in 250 ml of Hoagland solution (Wang, 1986), containing 20 μg/ml of diazinon in each 18 Erlenmeyer flask 500 ml. The same number of flasks with only pesticide solution (20 μg/ml) was prepared. Three flasks were prepared as a control with a plant alone. After 1, 3, 7, 10, 14 and 21 days, three exposed and three control plants were randomly harvested. Plant roots were rinsed in running tap water for 2 minutes and were blotted dry. The plants were dissected into individual leaves and roots then 5 g of leaves and 3 g of roots were analyzed for the pesticide residues.

Residues Analysis

Soil samples were extracted and cleaned up according to the method of Krause *et al.*, 1986. Soil samples (20 g) were shaken mechanically with 50 ml of acetone-water (3:1) for one hour in 500 ml glass stopper bottle. The extract was filtered through a clean pad of cotton, and then, 50 ml of filtrate was concentrated by using a rotary evaporator in a water bath at 40°C to remove acetone and then extracted twice with 50 ml chloroform. The combined chloroform extract was dried using anhydrous sodium sulfate and then evaporated to dryness at 40°C using a rotary evaporator for GC determination. Water samples were extracted with methylene chloride without clean up using a continuous liquid-liquid extraction (El-Sheamy *et al.*, 1991). Diazinon was extracted from the root, stalk and leaf samples with acetone or water acetone and then extracting with petroleum ether and dichloromethane. The organic phase was separated, dried, and concentrated just to dryness (Luke *et al.*, 1981). Samples of soil, water, root, stalk and leaf extracts were analysed for diazinon determination by use of a Agilent model 6890 gas chromatograph equipped with flame photometric detector (FPD) with phosphorus filter. A fused silica capillary (PAS-1701) column containing 14% cyanopropilsyloxane as stationary phase (30 m length x 0.32 mm internal diameter (i.d) x 0.25 μm film thickness), was used for the separation in the GC. Nitrogen was used as carrier gas at 3 ml/min, pulsed splitless mode. The GC temperature for oven was 220°C. The detector and injector temperature were set at 250°C and 240°C, respectively. The detector gas (hydrogen) flow was 75 ml/min, the air flow was 100 ml. $min.^{-1}$.

Data Analysis

The rate of degradation (k) and half-life (t 1/2) were calculated according to the method described with Gomaa and Belal, 1975.

RESULTS AND DISCUSSION

Kinetics of Pesticide Removals in Soil Experiment

Data obtained during the present investigation revealed that all experimental sets containing plants removed a substantial amount of diazinon. As shown in Table 1, diazinon concentrations in soil with *P. major*, and *H. annus* reduced by 48.20–99.55 % and 17.90–99.00 %, respectively throughout 1- to 21-days exposure, compared with 14.60–53.65% in the soil of control (Fig.1). The half-life value ($t_{1/2}$) of diazinon, calculated by first-order reaction, for soil with *P. major* and *H. annus* was found to be 4.51 and 7.05 days, respectively, compared with 21.40 days for soil alone (Table 1). Results with the disappearance rate constant (k_r) values revealed that diazinon had the highest k_r value and shortest $t_{1/2}$ in all experimental sets containing plants, while diazinon had the lowest k_r and longest $t_{1/2}$ in unplanted soil. These data demonstrated that most of the diazinon disappearance by the tested two plants in soil may be attributed to the uptake potential and transformation or degradation by the enzyme induction capability of the plant or by microorganisms in the plant root zone. Only one of them contributes to the reduction of a contaminant or connected them (Deepali *et al.*, 2009; Yao *et al.*, 2009).

The Role of two Plants in Diazinon Uptake and Translocation in Soil Experiment

Diazinon concentrations (μg/g) in different parts of *P. major* and *H. annus* are shown in Fig. 2a–c. Root concentrations of the diazinon were always higher than those of the leaves of the two plants. Diazinon accumulated in the roots of *P. major* to reach the maximum levels after one day (14.77 μg /g). Afterwards, concentration decreased gradually throughout the test. While, in *H. annus* roots reached the maximum after 10 days of exposure (16.40 μg/g), then decreased until the end of testing (Fig. 2a). *P. major* roots

Table 1. Phytoremediation of diazinon in soil planted with *Plantago major* L. and *Helianthus annus* L.

Treatments	Days after application								
	1	3	7	10	14	21	$T_{1/2}$ (per days)	K_r (per days)	AUC_s (per days)
In soil									
µg/g	17.08	16.82	15.29	13.11	11.09	9.27	21.40	0.032	277.31
Loss (%)	14.60	15.95	23.50	43.45	44.50	53.65			
In soil with *P. major*									
µg/g	10.36	8.27	4.63	2.57	0.97	0.09	4.51	0.15	78.05
Loss (%)	48.20	58.65	76.80	87.10	95.15	99.55			
In *P. major* roots									
µg/g	14.77	12.51	9.96	7.60	5.20	1.81			
In *P. major* leaves									
µg/g	2.33	8.08	4.75	1.25	0.74	0.37			
Total uptake	17.10	20.59	14.71	8.85	5.94	2.18			
In soil with *H. annus*									
µg/g	16.42	14.51	10.53	8.58	4.02	0.20	7.05	0.09	169.34
Loss (%)	17.90	27.40	47.30	57.10	80.00	99.00			
In *H. annus* roots									
µg/g	3.66	8.65	11.23	16.40	12.51	8.95			
In *H. annus* stalks									
µg/g	0.96	1.96	2.48	3.69	11.94	5.16			
In *H. annus* leaves									
µg/g	0.81	1.40	1.85	2.73	6.28	5.23			
Total uptake	5.43	12.01	15.56	22.82	30.73	19.34			

$T_{1/2}$, half-life; k_r, disappearance rate constant; AUCs, areas under the curve represent compound concentration during the period of study

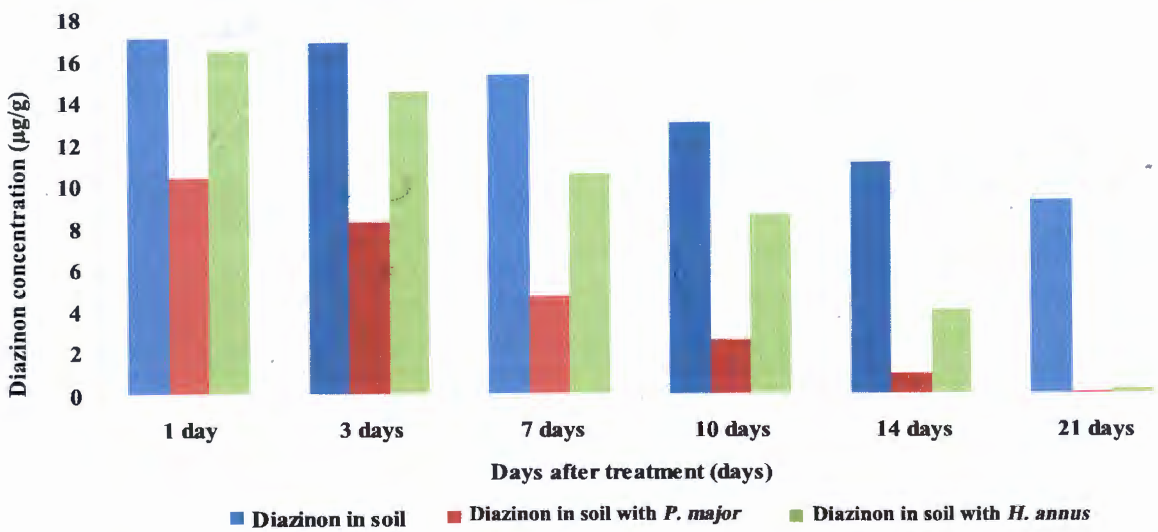

Fig. 1. Degradation of diazinon in soil planted with two species of plants, *Plantago major* L. and *Helianthus annus* L.

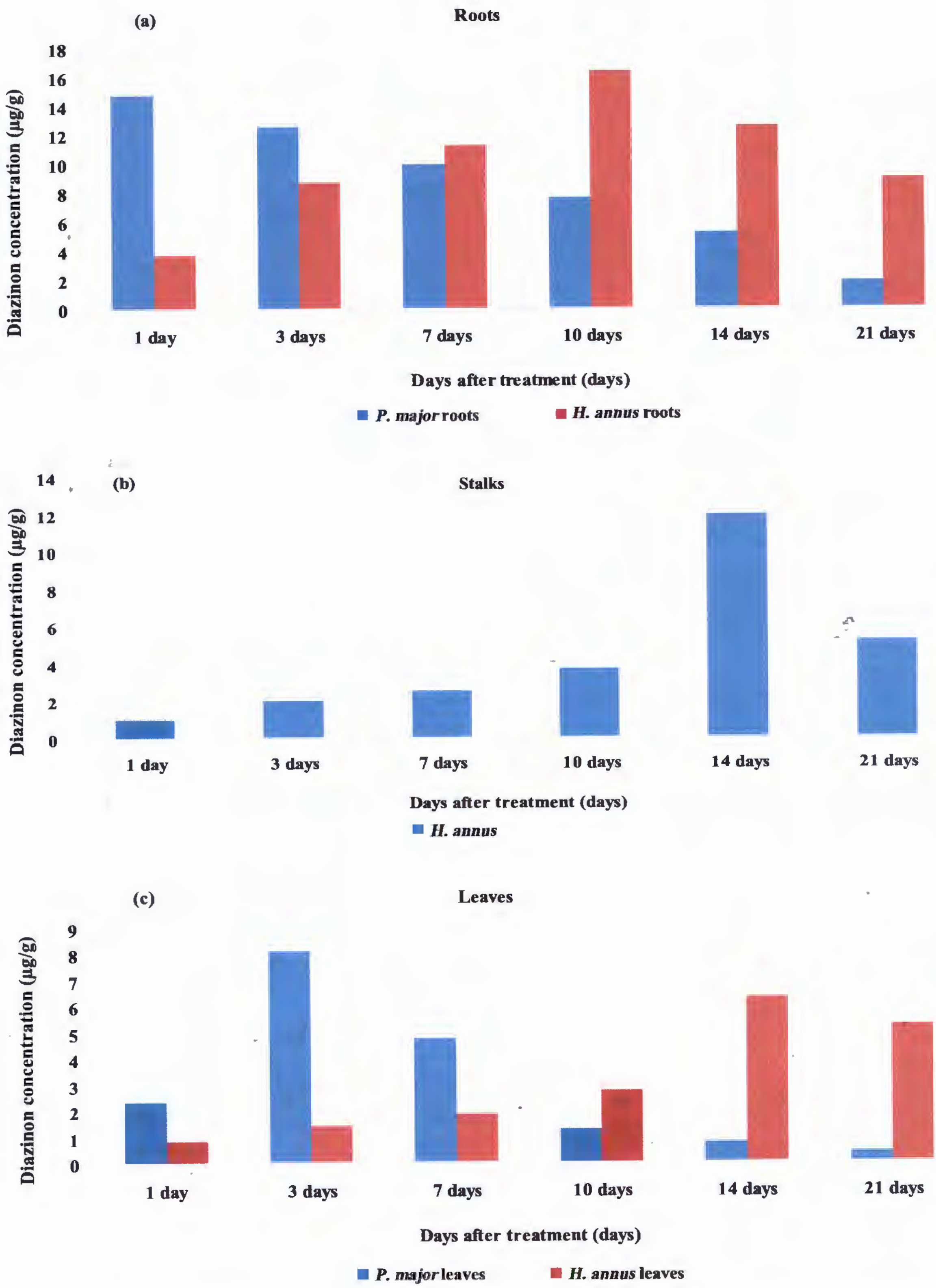

Fig. 2. Uptake and translocation of diazinon using two species of plants, *Plantago major* L. and *Helianthus annus* L. in soil (a) diazinon in roots, (b) diazinon in stalks, (c) diazinon in leaves

were an efficient accumulator of diazinon in short time while *H. annus* roots were an efficient accumulator of diazinon in long time.

Diazinon was translocated into *P. major* leaves much faster than *H. annus* leaves. In the leaves, diazinon translocated into the *P. major* leaves and reached the maximum after 3 days of exposure (8.08 µg/g) then decreased until the end of testing, while it reached the maximum after 14 days of exposure (6.28 µg/g) in *H. annus* leaves then decreased until the end of experiment (Fig. 2c). In the stalks of *H. annus*, diazinon was accumulated and reached the maximum after 14 days of exposure (11.94 µg /g) then decreased until the end of experiment (Fig. 2b). *H. annus* showed an effect on uptake of diazinon into roots, and the uptake ratio was about 1.11 times higher when compared with *P. major*, while *P. major* showed an effect on translocation of diazinon into leaves, and the uptake ratio was about 1.28 times higher when compared with *H. annus*. It has been observed that roots were important in accumulating compounds due to their direct exposure to toxic chemicals with underground parts, and transporting the compounds to above-ground organs, shoots (Azmat *et al.*, 2009). The uptake and translocation of organic compounds are dependent on hydrophobicity (lipophilicity), solubility, polarity, molecular weight, plant species and environmental factors (Turgut, 2005). Lipophilicity is the most important property of a chemical in determining its movement into and within a plant and is related to the n-octanol/water partition coefficient (K_{ow}) value. For uptake, log K_{ow} must be typically between 0.5 and 3.0. Compounds with larger log K_{ow} are hydrophobic and may adsorb strongly into roots, while smaller log K_{ow} are too hydrophilic to pass through cell membrane (Bouldin *et al.*, 2006). Diazinon has a log K_{ow} of 3.3, vapor pressure of 1.4×10^{-4} mm Hg at 20°C, and Henry's law constant of 1.4×10^{-6} atm m^3/mol which would indicate that it would not easily volatilize from soil or water (Howard, 1991). Diazinon is expected to quickly cross biomembranes and then sorb to the roots (Evans *et al.*, 1998), while the root uptake and translocation of diazinon to above-ground biomass of plants is low; therefore, diazinon residue accumulated in the roots more than that the aerial part of the plant organs. It has been observed that roots were important in accumulating compounds due to their direct exposure of toxic chemicals with underground parts, and transporting the compounds to above-ground organs (leaves) (Azmat *et al.*, 2009). Plants can accumulate or metabolize a variety of organic compounds, including, chlorpyrifos (Wang *et al.*, 2007; Lytle and Thomas, 2009; Prasertsup and Naiyanan 2011; Romeh and Hendawy, 2013), methyl parathion (Khan *et al.*, 2011) and cyanophos (Romeh, 2014). *Glycine max* L. roots were an efficient accumulator of azoxystrobin (25.32 mg/kg), followed by *P. major* roots (20.62 mg/kg) and *H. annus* roots (18.29 mg/kg), within 10 days, respectively. In the leaves, azoxystrobin significantly translocated into the *P. major* leaves and reached the maximum after 10 days of exposure (15.03 mg/kg), followed by *H. annus* leaves (9.8 mg/kg), while it reached the maximum after 3 days of exposure (3.12 mg/kg) in *G. max* leaves (Romeh, 2015).

Kinetics of Pesticide Removals in Water Experiment

Table 2 show the phytoremediation potential of *P. major* to remove diazinon insecticide from contaminated water. Viable whole plant of *P. major* in water solution reduced diazinon residues by 32.35–95.40 % during 1- to 21-day of exposure periods as compared with 6.90–68.30 % in water solution without the plantain (Table 2). The half-life value ($t_{1/2}$) of diazinon, calculated by first-order reaction, for water planted with *P. major* and in unplanted water was found to be 3.02 and 16.30 days, respectively (Table 2). Viable whole broadleaf plantain in water solution reduced imidacloprid residues by 55.81–95.17%, during 1–10 days of exposure periods compared with 13.71–61.95% in water solution without the plantain (Romeh, 2010). The half-life value ($t_{1/2}$) of cyanophos, calculated by first-order reaction, for water planted with *P. major* and in unplanted water was found to be 1.73 and 13.63 days, respectively (Romeh, 2014). The disappearance rate constant (k_r) is 5.5 times greater in water solution with plantain than in the diazinon control. The areas under the curve (AUC) represent compound concentration during the period of study. AUC in the water solution with plantain was decreased than diazinon control (Table 2).

Table 2. Phytoremediation of diazinon in water planted with *Plantago major* L.

Treatments	Days after application								
	1	3	7	10	14	21	$T_{1/2}$ (per days)	K_r (per days)	AUC_s (per days)
In water solution									
µg/ml	18.62	17.49	15.53	14.87	11.76	6.34	16.30	0.04	280.93
Loss (%)	6.90	12.50	22.30	25.60	41.20	68.30			
In water with *P. major*									
µg/ml	13.53	5.77	4.89	1.34	1.08	0.92	3.02	0.22	86.48
Loss (%)	32.35	71.15	75.55	93.30	94.50	95.40			
In *P. major* roots									
µg/g	37.80	32.78	28.82	22.19	17.69	13.85			
In *P. major* leaves									
µg/g	3.46	5.73	10.51	4.87	3.97	2.09			
Total uptake	41.26	38.51	39.33	27.06	21.66	15.94			

$T_{1/2}$, half-life; k_r, disappearance rate constant; AUCs, areas under the curve represent compound concentration during the period of study

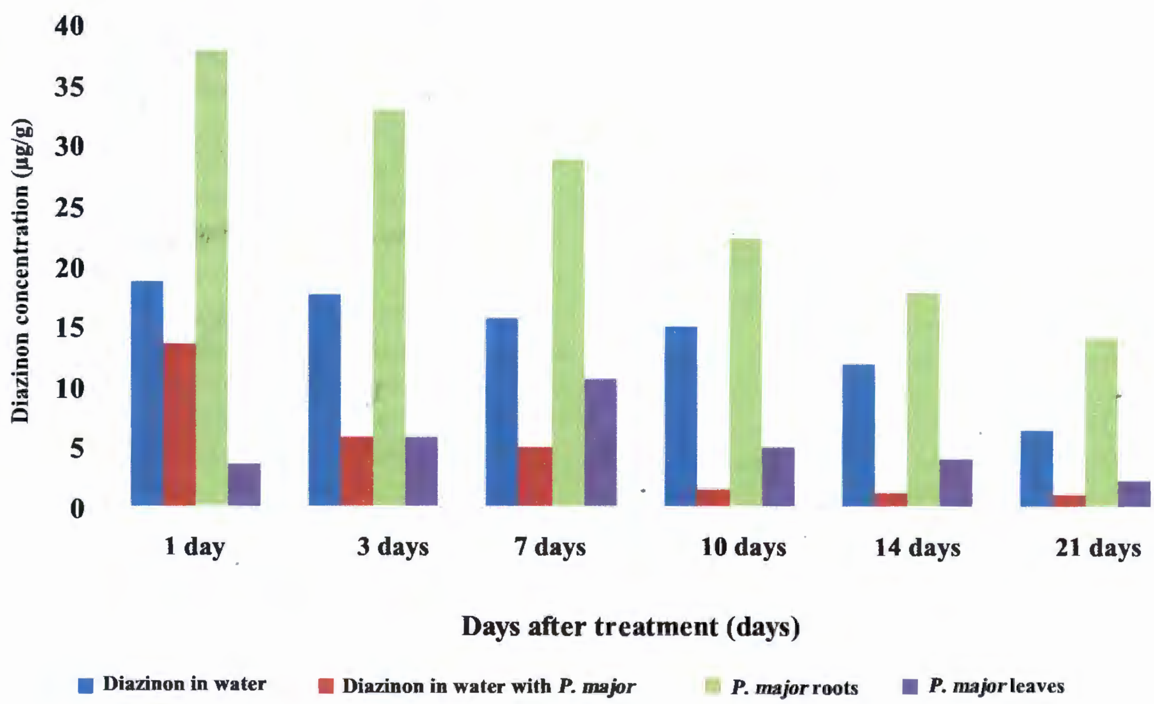

Fig. 3. Uptake and translocation of diazinon by *Plantago major* L. in water solution

The Role of *P. major* in Diazinon Uptake and Translocation in Water Experiment

Diazinon was taken up by roots much faster than leaves. In water, diazinon accumulated in the roots of *P. major* to reach the maximum levels after one day (37.80 µg/g). Afterwards, concentration decreased gradually throughout the test. Diazinon translocated into the leaves (3.46 µg/g) within one day and reached the maximum after 7 days of exposure (10.51 µg/g), then decreased until the end of testing (Table 2). Several grass species were utilized to create grass of waterways and buffer strips to contain polluted surface waters (Rankins *et al.*, 2001; Zhao *et al.*, 2003). Most of the transport of pesticides occurs in aquatic plants *via* rhizomes. Once moved inside the plants, pesticides are distributed from the roots primarily into the leaves and lost *via* diffusion if volatile (Karthikeyan *et al.*, 2004). The rooted portion of *Acorus gramineus* plays an import role in the total pesticide-uptake due to the relatively high weight-based accumulation of pesticides and its contribution to organ biomass proportion (Chuluun *et al.*, 2009). In water with plantain, cyanophos significantly accumulated in plantain roots and leaves to reach maximum levels after two and four hours of treatment, respectively. After one day, the concentration of cyanophos decreased in roots and shoots until the end of testing (Romeh, 2014).

Conclusion

In conclusion, our study found that *P. major* and *H. annus* were the most suitable plant species for phytoremediation of diazinon-contaminated soil and water.

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المعالجة النباتية للتربة والمياه الملوثة بالديازينون

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يعتبر الديازينون واحداً من أكثر المبيدات الفوسفورية الحشرية المستخدمة ضد آفات مختلفة، وقد يؤدي استخدام المبيد على المجموع الخضري لتراكم متبقياته في التربة وغسيلها مع الماء السطحي ثم دخولها للماء الأرضي، وتعتبر المعالجة النباتية واحدة من أكثر الطرق الواعدة لتجنب المخاطر الناجمة عن مثل هذا التلوث، ويستهدف هذا العمل دراسة كفاءة نباتين في إزالة متبقيات مبيد الديازينون من التربة والماء، تم اختبار نباتي لسان الحمل (*Plantago major* L.) ودوار الشمس (*Helianthus annus* L.) لتنقية التربة وكذلك استخدام نبات لسان الحمل لتنقية المياه تحت الظروف المعملية، أوضحت النتائج المتحصل عليها أن الديازينون الممتص في جذور نبات لسان الحمل ودوار الشمس كان أكثر من الأوراق، في تجارب التربة كان تناول الديازينون في جذور لسان الحمل ليصل إلى أعلى مستوياته بعد واحد يوم (١٤,٧٧ ميكروجرام/ جم) ثم تناقص تدريجياً أثناء التجربة، بينما تناول جذور دوار الشمس قد وصلت لأعلى مستوياتها بعد عشرة أيام من التعرض (١٦,٤٠ ميكروجرام/ جم) ثم تناقصت حتى نهاية التجربة، كانت جذور لسان الحمل أكثر كفاءة في تجميع الديازينون في وقت قصير بينما تجمع المركب في دوار الشمس فى وقت أطول، انتقل الديازينون في أوراق لسان الحمل أسرع من أوراق دوار الشمس، في المياه تجمع الديازينون في جذور لسان الحمل ليصل أقصاه بعد واحد يوم (٣٧,٨٠ ميكروجرام/جم) بينما انتقل إلى الأوراق ووصل أقصى مستوياته خلال سبعة أيام من المعاملة (١٠,٥١ ميكروجرام/جم)، وتشير النتائج إلى أن نبات لسان الحمل ذو قدرة عالية في التخلص من متبقيات الديازينون في المياه والتربة وأنه يمتلك نشاط واسع في المعالجة النباتية للمبيدات.

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