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NOVEL PHYTO PLANT OF POP-PESTICIDES: ENERGY CROP *MISCANTHUS SINENSIS*

The collapse of agricultural infrastructure in Kazakhstan and other countries worldwide has resulted in the transfer of ownership or abandonment of plant protection chemicals storage facilities. Despite legislative measures and international conventions, the issue of persistent organic pollutants (POPs) remains unresolved. The measures to restore POP-pesticide-contaminated areas present limited options. Hence phytotechnology emerges as a promising strategy due to eco-friendliness and the absence of significant capital investments. One of the key aspects of technology is the search for novel plant species capable of accumulating and transforming these contaminants into less toxic compounds. Current study investigated the potential of the energy crop *Miscanthus sinensis* Anderson (*M. sinensis*) as a novel phyto plant for remediating POP-pesticide-contaminated soils. The experimental layout comprised cultivation of *M. sinensis* in POP-pesticide-contaminated (2.4-DDD, 4.4-DDD, 4.4-DDE, 4.4-DDT, α-HCH, β-HCH, γ-HCH, and δ-HCH) soil. Biomass productivity, physiological parameters, and phytoremediation potential were assessed at harvest. Our findings revealed that POP-pesticides influenced productive and physiological parameters of *M. sinensis* differently, specifically: reduced aboveground biomass and chlorophyll pigments content by up to 23 and 37%, respectively, and increased root biomass by up to 17%. Furthermore, the plant exhibited a remarkable tolerance to severe POP-pesticide contamination, as evidenced by a tolerance index of 0.99. Evaluation of phytoremediation coefficients revealed that *M. sinensis* employed distinct strategies depending on POP-pesticide: phytoextraction and phytostabilisation. 4.4-DDT, β-HCH, and γ-HCH were accumulated in aboveground biomass with translocation factors of 1.18, 4.04, and 84.0, respectively. Whereas metabolite 4.4-DDE was accumulated in plant roots with a bioconcentration factor of 2.07. Study results suggest that *M. sinensis* holds great promise for use in POP-pesticides phytoremediation projects, particularly in Kazakhstan, owing to confirmed phytostabilisation activity concerning 4.4-DDE, the final metabolite of 4.4-DDT degradation. Therefore, further research should focus on optimizing *M. sinensis* phytostabilisation strategies for other POP-pesticides.

Key words: energy crop; *Miscanthus sinensis*; productivity; photosynthesis; phytoremediation; soil; POP-pesticides.

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ТОЛ-пестицидтерге арналған жаңа фиторемедиант: *Miscanthus Sinensis* энергетикалық өсімдік

Қазақстан және басқа да көптеген елдерде ауыл шаруашылық инфрақұрылымының ыдырауына байланысты өсімдіктерді қорғауға арналған химиялық заттарды сақтайтын қоймалар, сондай-ақ оларда сақталатын препараттардың қалдықтары жеке меншікке өтті немесе иесіз болып қалды. Заңнамалық актілерге және халықаралық конвенцияларға қарамастан, ескірген пестицидтер, оның ішінде тұрақты органикалық ластаушы заттардың (ТОЛ) мәселесі толық шешілмеген. ТОЛ-пестицидтерімен ластанған аумақтарды қалпына келтіру шаралары шектеулі, сондықтан фитотехнология өзінің экологиялық тазалығы мен күрделі қаржы жұмсалымының болдырмауына байланысты болашағы зор технология болып табылады. Технологияның маңызды кезеңдерінің бірі – пестицидтерді жинақтап, аз уытты қосылыстарға айналдыра алатын жаңа өсімдік түрлерін табу. Мақалада ТОЛ-пестицидтерімен ластанған топырақты қалпына келтіру үшін жаңа дақыл ретінде *Miscanthus sinensis* Anderson (*M. sinensis*) энергетикалық дақылының потенциалы қарастырылды. Эксперименттік жоба ТОЛ-пестицидтерімен (2.4-ДДД, 4.4-ДДД, 4.4-ДДЕ, 4.4-ДДТ, α-ГХЦГ, β-ГХЦГ, γ-ГХЦГ және δ-ГХЦГ) ластанған топырақта *M. sinensis*-ті өсіруді қамтиды. Вегетациялық кезең аяқталғаннан кейін биомассаның өнімділігі, физиологиялық параметрлері және фиторемедиациялық потенциалы бағаланды. Алынған нәтижелер бойынша ТОЛ-пестицидтер *M. sinensis*-тің өнімділігімен физиологиялық көрсеткіштерге: жер үсті биомассасының мен хлорофилл пигментерінің құрамы төмендеуі, 23 және 37%-ге дейін сәйкесінше, тамыр жүйесінің биомассасы 17%-ға дейін өскенін көрсетті. Өсімдік ТОЛ-пестицидтермен ластануға төзімділігін көрсетті, төзімділік индексі 0.99 құрады. Фиторемедиация көрсеткіштерін бағалау кезінде *M. sinensis* ТОЛ-пестицидінің түріне байланысты ластанған топырақты қалпына келтірудің екі стратегиясын қолданғаны анықталды: фитоаккумуляция және фитотұрақтандыру. 4.4-ДДТ және β-ГХЦГ және γ-ГХЦГ изомерлері ластанған топырақтан жерүсті бөлігінде жинақталды, транслокация коэффициенті 1.18, 4.04 және 84.0, сәйкесінше; 4.4-ДДЕ метаболиті тамыр жүйесінде жинақталды, биоконцентрация коэффициенті 2.07. Бұл зерттеудің нәтижелері, фитотұрақтандырғыш белсенділігімен 4.4-ДДТ ыдырауының соңғы метаболиті 4.4-ДДЕ улы метаболиттің өзара байланыстың расталғандықтан *M. sinensis*-тің ТОЛ-пестицидтерін фиторемедиациялау жобаларында қолдану, әсіресе Қазақстанда, үлкен потенциалға ие екенін көрсетеді. Сондықтан, одан әрі зерттеулерде *M. sinensis* фитотұрақтандыру стратегиясын оңтайландыру басқа ТОЛ-пестицидтерге қарсы бағытталуы керек.

Түйін сөздер: энергетикалық өсімдік; *Miscanthus sinensis*; өнімділік; фотосинтез; фиторемедиация; топырақ; ТОЛ-пестицидтер.

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Новый фиторемедиант СОЗ-пестицидов: энергетическое растение *Miscanthus Sinensis*

С развалом сельскохозяйственной инфраструктуры в Казахстане и во многих других странах хранилища химических средств защиты растений, как и хранящиеся в них остатки препаратов, перешли в частное владение, либо оказались бесхозными. Несмотря на законодательные акты и международные конвенции проблема стойких органических загрязнителей (СОЗ) до конца не решена. Меры восстановления территорий, загрязненных СОЗ-пестицидами, ограничены, в связи с этим фитотехнология является многообещающей благодаря экологичности и отсутствию крупных капиталовложений. Одним из ключевых моментов технологии является поиск новых видов растений, способных накапливать и трансформировать пестициды в менее токсичные соединения. В статье рассматривается потенциал энергетической культуры *Miscanthus sinensis* Anderson (*M. sinensis*), как новой культуры, для восстановления почв, загрязненных СОЗ-пестицидами. Дизайн эксперимента включает культивирование *M. sinensis* на загрязненной СОЗ-пестицидами (2,4-ДДД, 4,4-ДДД, 4,4-ДДЕ, 4,4-ДДТ, α-ГХЦГ, β-ГХЦГ, γ-ГХЦГ и δ-ГХЦГ) почве. Продуктивность биомассы, физиологические параметры и фиторемедиационный потенциал оценивали по окончании вегетационного периода. Результаты показали, что СОЗ-пестициды оказывали влияние на продуктивные и физиологические показатели *M. sinensis*: снижали наземную биомассу и содержание пигментов хлорофилла до 23 и 37%, соответственно, и повышали биомассу корневой системы до 17%. Растение продемонстрировало устойчивость к загрязнению СОЗ-пестицидами, индекс толерантности составил 0.99. При оценке фиторемедиационных показателей выявлено, что *M. sinensis* в зависимости от вида СОЗ-пестицида использовал две стратегии восстановления загрязненной почвы: фитоэкстракцию и фитостабилизацию. 4,4-ДДТ и изомеры β-ГХЦГ и γ-ГХЦГ аккумулировались в наземной биомассе, коэффициенты транслокации – 1.18, 4.04 и 84.0, соответственно; метаболит 4,4-ДДЕ накапливался в корневой системе, коэффициент биоконцентрации – 2.07. Результаты исследования позволяют предположить, что *M. sinensis* имеет большие перспективы для использования в проектах по фиторемедиации СОЗ-пестицидов, особенно в Казахстане, благодаря подтвержденной фитостабилизирующей активности в отношении токсичного 4,4-ДДЭ, конечного метаболита разложения 4,4-ДДТ. Поэтому дальнейшие исследования должны быть сосредоточены на оптимизации стратегии фитостабилизации *M. sinensis* в отношении других СОЗ-пестицидов.

Ключевые слова: энергетическое растение; *Miscanthus sinensis*; продуктивность; фотосинтез; фиторемедиация; почва; СОЗ-пестициды.

Introduction

Persistent Organic Pollutants (POPs), particularly organochlorine pesticides (OCPs), are a long-lasted concern in environmental matrices worldwide. The danger posed by POPs led to the adoption of the Stockholm Convention in 2001, which Kazakhstan signed on May 23, 2001, and ratified on June 7, 2007. The convention aims to reduce the production and use of POPs, recognized for their harmful effects on humans and their ability to travel long distances. Initially, the convention listed 12 chemicals, including 9 pesticides (DDT, aldrin, dieldrin, endrin, chlordane, heptachlor, mirex, toxaphene, and HCB). By 2013, this list had expanded to include 13 OCPs, with additions in 2009 such as chlordecone, α-HCH, β-HCH, γ-HCH, and pentachlorobenzene [1].

Kazakhstan's recognition of the POP-pesticide problem started with identifying numerous anthropogenic activities leading to historical pollution. In 2008, around 10,000 tons of banned obsolete pesticides unsuitable were registered [2]. By 2012, 1,500 tons of obsolete pesticides and their mixtures across the country along with 602 pesticide storage facilities were recorded [3,4]. Nowadays, according to the annual environmental monitoring of the Republic of Kazakhstan, different environmental matrices are regularly being identified as contaminated with pollutants of diverse origin [5]. Recent inventories revealed 727 pesticide storage facilities and 5 operating landfills containing approximately 2,101 tons of obsolete pesticides in Kazakhstan [6,7].

A critical characteristic shared by all POP-pesticides is their pronounced lipophilicity, leading to their biomagnification in organs like the liver and adipose tissue and causing significant detrimental effects on human health [8–10]. In Kazakhstan, prevalent POP-pesticides in soil are classified into three categories: dichlorodiphenylethanes, chlorineted benzenes and cyclohexanes, and chlorinated cyclodienes [11]. The most common POP-pesticides in soils near former pesticide storage facilities include DDT, its metabolites, and HCH isomers.

Hence, there is a pressing need for ecologically benign remediation approaches [12], with phytoremediation emerging as a promising solution that applies plants to uptake, accumulate, and detoxify contaminants from environmental matrices [9,11,13–16]. Energy crops have emerged as exceptionally promising tools for phytoremediation, offering a dual benefit of environmental cleanup and economic revenue generation through biomass production [17–19]. Their biomass serves as a valuable resource for biofuel production, as well as raw materials for various industries, including construction, insulation, and paper production. One energy crop that has gained significant recognition for its phytoremediation prowess is *Miscanthus* × *giganteus* Greef et Deu [20,21]. It thrives on marginal soils for extended periods, typically spanning 20-25 years. However, it has a notable limitation when it comes to tolerance for POP-pesticides, maxing out at just twice the Maximum Permissible Concentration (MPC), equivalent to ~200 μg kg^{-1} [13]. In the quest for a more robust phytoremediation solution, attention has turned to *Miscanthus sinensis* Anderson, a perennial C_4 energy crop with remarkable productivity, yielding biomass at rates of up to 36.6 t DM ha^{-1} yr^{-1} [22]. More importantly, the crop was found to tolerate superior POP-pesticide contamination, up to 62×MPC [13,14]. Given its ability to grow in organically contaminated soil, *M. sinensis* emerges as a promising candidate for the remediation of POP-pesticides contaminated soils, especially in Kazakhstan, being not indigenous and having the potential for large-scale recultivation.

Thus, the current study aimed to investigate the biomass productivity and phytoremediation potential of *M. sinensis* grown in historically POP-pesticides contaminated soil collected in the vicinity of a former pesticide storage facility.

Materials and methods

2.1 Soil collection

Two distinct soil types were employed in the experiment, specifically: POP-pesticides contaminated soil collected in the vicinity of a former obsolete pesticide storage facility in Kyzylkairat village (GPS 43°17'58.7" N 77°11'39.6" E), district, Almaty region, Kazakhstan; and a background, hereafter referred as “control soil”, collected at the base of Peak Talgar (GPS 43°16'36" N, 77°12'37" E), Talgar district, Almaty region, Kazakhstan. Soil collection was performed following ISO 18400-205:2018 [23], in particular the ‘envelope’ method: five soil samples were taken from a 5 × 5 m test square at a depth of 0-0.6 m. Subsequently, the soil samples were sieved (d = 3 mm) to remove plant debris and stones, thoroughly homogenized, air-dried, sampled for agrochemical and chemical analyses [24,25], and stored at a temperature of 4℃ until the experiment establishment.

According to the World Reference Base for Soil Resources classification [26], research soils belong to chernozem with a density of 1.44 g cm^{-3}. According to the soil agrochemical profile (Table 1), POP-pesticides contaminated soil demonstrated significantly higher concentrations of mobile phosphorus and potassium compared to control soil. Whereas control soil was rich in organic matter and nitrogen content (Table 1).

Table 1 – Agrochemical profiles of the research soils.

Parameter	Unit	Control soil	Contaminated soil	Measuring standard
Organic matter, C	%	34.0 ± 1.45 a	6.10 ± 0.02 b	Tyurin method [27,28]
pH (H_2O)	-	7.34 ± 0.08 b	7.85 ± 0.02 a	GOST 26423-85 [29]
Total N	mg kg^{-1}	432 ± 5.51 a	96.7 ± 7.20 b	Tyurin & Kononova method [30]
P_2O_5	mg kg^{-1}	230 ± 10.0 b	400 ± 5.00 a	Machigin method in CINAO modification [31]
K_2O	mg kg^{-1}	440 ± 40.0 b	885 ± 25.0 a	
Ca	mEq/100 g	58.3 ± 2.45 a	20.8 ± 0.75 b	Arinushkin method in Grabarov modification
Mg	mEq/100 g	8.37 ± 0.45 a	3.70 ± 0.23 b	
Na	mEq/100 g	0.28 ± 0.01 b	0.38 ± 0.01 a	Antipov-Karataev & Mametov method in Grabarov modification
K	mEq/100 g	0.27 ± 0.01 b	1.04 ± 0.03 a	

Notes: different letters within one parameter indicate a statistical difference at $p < 0.05$.

Table 2 – POP-pesticides concentrations (μg kg^{-1}) in historically contaminated soil.

POP-pesticide	MPC		Contaminated soil
	KZ [32]	**EU** [33]	
DDT and metabolites			
2.4-DDD	-	-	14 072 ± 5 239
4.4-DDD	-	-	11 434 ± 7 302
4.4-DDE	-	-	778 ± 292
4.4-DDT	-	-	10 023 ± 2 471
∑ DDTs	**100**	**10.0**	**36 307**
HCH isomers			
α-HCH	-	220	89.2 ± 0.0
β-HCH	-	92.0	25.5 ± 16.4
γ-HCH	-	0.01	488 ± 152
δ-HCH	-	-	67.4 ± 13.7
∑ HCHs	**100**	**-**	**670**

2.2 Experimental layout

To assess the biomass productivity and phytoremediation potential of *Miscanthus sinensis* Andersson in POP-pesticide contaminated soil, a controlled pot experiment was conducted in greenhouse conditions.

The preparatory phase of the experiment commenced on November 13, 2019, entailing a structured process for filling the pots. Initially, 1 kg of keramzite was placed at the base of each pot, serving as an effective drainage layer. This was followed by a second layer of 1 kg of sand. The third layer consisted of 2 kg of soil, differentiated across pots as either POP-pesticides contaminated soil or control soil. To mitigate soil desiccation, a final thin layer of sand was uniformly applied atop each pot. In total, the experimental design incorporated 6 (six) pots, arranged to facilitate 2 (two) experimental variants, each replicated thrice.

On November 14, 2019, *M. sinensis* rhizomes were planted in prepared pots to facilitate plant acclimatization and adaptation during the winter period. The rhizomes were sourced from the plantation located on the premises of the Institute of Plant Biology and Biotechnology (GPS 43°13'38.161"N, 76°54'59.443"E; Almaty, Kazakhstan).

Plant physiological parameters, including plant height, length, width, and number of leaves, were measured monthly. Soil moisture was adjusted to 50% by irrigation every third day.

At the end of the vegetation season (September 15, 2020) when leaves turned yellow, *M. sinensis* biomass was harvested. The collection of soil and plant samples, comprising both roots and aboveground biomass (AGB), was performed following GOST 17.4.4.02-2017 [24] and ISO 18589-2:2022 [34]. The soil samples were dried and sieved (d = 2 mm). The roots of *M. sinensis* were thoroughly cleansed under running tap water to eliminate residual soil particles. Then, plant samples were dried at a temperature of 105 °C until a constant weight was achieved. The dried roots and AGB samples were finely using the IKA A11 basic analytical mill and stored at room temperature in labelled zip-lock bags until chemical analysis.

2.3 Chlorophyll pigments content

The content of chlorophyll pigments, specifically chlorophyll *a* (*Chl a*), chlorophyll *b* (*Chl b*), and carotenoids (*Car*), in the leaves of *M. sinensis* was determined according to Gavrilenko et al. [35]. 30 g of fresh leaves were finely ground in 2 mL of cooled 96% ethanol. Then, the produced homogenate was subjected to centrifugation at 7,000 rpm for 10 min. The supernatant was carefully transferred into a test tube. The absorbance levels of photosynthetic pigments within the supernatant were measured using an Evolution 60 spectrophotometer (Thermo Scientific, USA) at wavelengths of 440.5, 649, and 665 nm. The concentrations of *Chl a*, *Chl b*, and *Car* were calculated employing eq. 1-4:

$$Chl_a(mg\ L^{-1}) = 11.63 \times D_{665} - 2.39 \times D_{649} \quad (1)$$

$$Chl_b(mg\ L^{-1}) = 20.11 \times D_{649} - 5.18 \times D_{665} \quad (2)$$

$$Chl_{a+b}(mg\ L^{-1}) = 6.45 \times D_{665} + 17.72 \times D_{649} \quad (3)$$

$$Car\ (mg\ L^{-1}) = 4.695 \times D_{440.5} - 0.268 \times Chl_{a+b} \quad (4)$$

2.4 Chemical analysis

The concentrations of target POP-pesticides in soil and plant samples were measured by gas chromatography with an electron capture detector (Gas Chromatography Agilent Technologies 6890N) equipped with the autosampler Combi-PAL (CTC Analytics AG, Switzerland) in accordance with standards ST RK 2131-2011 [25] and ST RK 2011-2010 [36], respectively. A detailed description of the procedure was published earlier [14,15,37].

2.5 Phytoremediation potential

In order to evaluate the resilience of *M. sinensis* to POP-pesticide contamination as well as its phytoremediation potential, tolerance index (TI), bioconcentration factor (BCF), and translocation factor (TLF) were calculated following eq. 5-7 [38–41].

$$TI = \frac{[Plant\ growth\ parameter]\ in\ contaminated\ soil}{[Plant\ growth\ parameter]\ in\ control\ soil} \quad (5)$$

$$BCF = \frac{POP - pesticide\ concentration\ in\ plant\ tissue\ (\mu g\ kg^{-1})}{POP - pesticide\ concentration\ in\ soil\ (\mu g\ kg^{-1})} \quad (6)$$

$$TLF = \frac{POP - pesticide\ concentration\ in\ aboveground\ biomass\ (\mu g\ kg^{-1})}{POP - pesticide\ concentration\ in\ roots\ (\mu g\ kg^{-1})} \quad (7)$$

2.6 Statistical analysis

The data analysis was conducted using RStudio software (version 2023.06.0 Build 421, RStudio PBC, 2023). Tukey HSD tests were performed for the pairwise comparisons of the means, while ANOVA was used to confirm statistical significance. Subsequently, the treatments were categorised by letter in descending order, and graphs were generated. Significance was declared at $p < 0.05$.

Results and discussion

3.1 Influence of POP-pesticide contamination on M. sinensis biomass productivity

M. sinensis plants underwent a complete developmental cycle in both control and POP-pesticides contaminated soils. In March 2020, seedlings in control and contaminated soils exhibited average heights of 16.3 ± 0.5 and 16.0 ± 1.2 cm, respectively ($p = 0.68$). At harvest, the plants had attained heights of 48.2 ± 0.3 cm in control soil and 45.8 ± 0.5 cm in contaminated soil, with a slight but still significant ($p < 0.01$) decrease observed for plants grown in contaminated soil (Fig. 1a). Furthermore, a notable increase of 10.8 ($p < 0.001$) and 16.9% ($p < 0.01$) was observed in the roots' length and weight of *M. sinensis* grown in contaminated soil, respectively (Fig. 1b, c). Conversely, AGB DW of plants cultivated in POP-pesticides contaminated soil showed a substantial decrease of 23.1% ($p < 0.001$).

Consequently, the mean TI of *M. sinensis* when cultivated in POP-pesticides contaminated soil was computed to be 0.99. In particular, individual TI values were calculated to be as follows: the height TI – 0.95; AGB DW – 0.77; roots DW – 1.17; and roots length – 1.11. Thus, the ability of *M. sinensis* to maintain nearly normal growth and even enhanced root development under severe POP-pesticide contamination in soil cumulatively evidence the remarkable resilience of *M. sinensis* and its potential to be utilized as a promising phyto-agent in remediation projects.

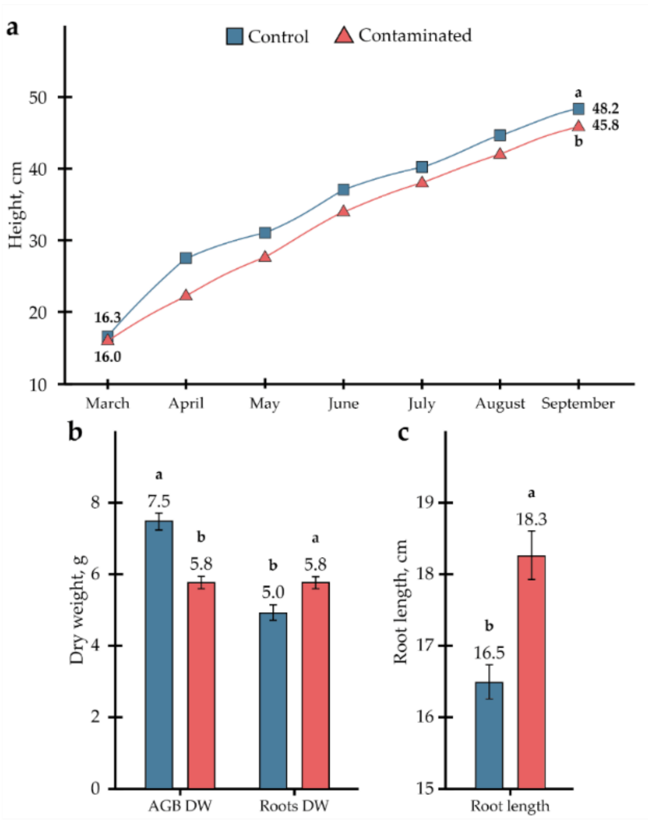

Figure 1 – Biomass productivity of *M. sinensis* grown in control and POP-pesticides contaminated soils: a) growth dynamic; b) AGB and roots DW; c) root length. Different letters within one parameter indicate a significant difference between values

3.2 Influence of POP-pesticide contamination on chlorophyll pigments content

Currently, research into indicators of plant tolerance to contaminants, identifying resilient plant species, and discovering species capable of accumulating toxic substances for phytoremediation is crucial. Hence, certain plant physiological parameters have emerged as essential bioindicators of abiotic stress caused by anthropogenic activities. These parameters include a decrease in the *Chl a/b* ratio and an increase in the *Chl (a+b)/Car* ratio. A notable reduction in the key photosynthetic pigment, *Chl a*, in response to an increase in auxiliary pigments such as *Chl b* and *Car*, indicates an adaptive response to xenobiotic stress [42].

In our study, the stress response of *M. sinensis* grown in POP-pesticides contaminated soil was evident in the reduction of *Chl a*, *Chl b*, and *Car* content by 30%, 37%, and 29%, respectively (Fig. 2a). This reduction suggests an adaptation of the plant's photosynthetic apparatus to severe POP-pesticide contamination. Consequently, we can infer that alterations in chlorophyll pigment ratios may serve as reliable indicators of the photosynthetic apparatus's adaptation to POP-pesticide exposure.

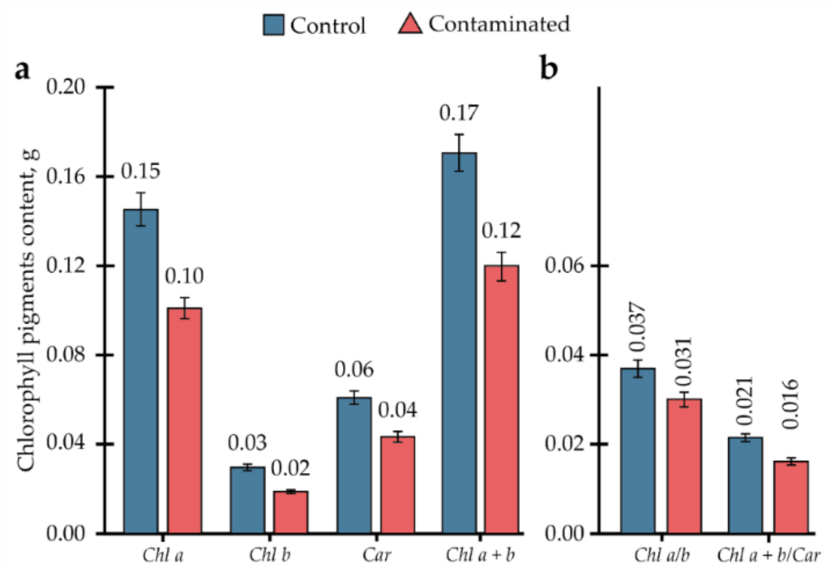

Figure 2 – Chlorophyll pigments content in leaves of *M. sinensis* grown in POP-pesticides contaminated soil: a) pigments mass; b) non-unit ratio indicators

3.3 Phytoremediation potential of M. sinensis concerning POP-pesticides

The molecular weights (M_r) of DDT & metabolites range from 318.0 to 354.5 g mol^{-1}. Notably, among DDT metabolites, 4.4-DDT exhibited the highest hydrophobicity coefficient ($log\ K_{ow}$) of 6.91 and the largest M_r at 354.5 g mol^{-1}. The $log\ K_{ow}$ for 4.4-DDE was slightly lower at 6.51, with an M_r of 318.0 g mol^{-1}. The lowest hydrophobicity coefficient was observed for 2.4-DDD ($log\ K_{ow}$ = 5.87) with a M_r of 320.0 g mol^{-1}, even though the $log\ K_{ow}$ for 4.4-DDD was higher at 6.02.

The accumulation of DDT & metabolites in *M. sinensis* tissues demonstrated a clear pattern: both 2.4-DDD and 4.4-DDD showed minimal concentration in the biomass, with bioconcentration factors (BCF) for AGB and roots being below 1 (Fig. 3a). This indicates a lack of significant accumulative capability in the plant for these pesticides. In contrast, the highest BCF was observed for 4.4-DDE, particularly in the root system, with a BCF of 2.07, while its BCF for AGB was 1.02 (Fig. 3a). According to the translocation factor (TLF) values, *M. sinensis* predominantly accumulated 4.4-DDE in the roots evidencing phytotabilization potential in relation to this POP-pesticide (Fig. 3b). Conversely, a slight phytoextraction potential was observed for 4.4-DDT (Fig. 3b).

Therefore, it can be inferred that *M. sinensis* able to bioconcentrate both 4.4-DDE and 4.4-DDT (BCF

>1), activating phytostabilization (TLF < 1) and phytoextraction (TLF > 1) strategy, respectively (Fig. 3a, b).

The M_r of HCH isomers (α-, β-, δ-, and γ-) range from 290.8 to 296.9 g mol^{-1}. Within this group, δ-HCH exhibits the highest hydrophobicity coefficient ($log\ K_{ow}$ = 4.14), while γ-HCH has the lowest (3.72), even though M_r of these isomers are identical.

The accumulation of HCH isomers in *M. sinensis* tissues predominantly followed the phytoextraction strategy: three out of four isomers were more concentrated in AGB than in the root system, with α-HCH being evenly distributed throughout the plant (Fig. 4a, b). However, BCF values indicate that *M. sinensis* cannot concentrate α- and δ-HCH (BCF < 1). Among these POP-pesticides, the highest accumulation was observed for β-HCH, which had a BCF of 6.55 in the AGB and 1.62 in the root system, accompanied by a TLF of 4.04. On the other hand, γ-HCH, due to its lower hydrophobicity, predominantly migrated from the roots to the AGB (TLF = 84), resulting in significant accumulation in the latter. Consequently, *M. sinensis* demonstrated a capacity to bioconcentrate the less toxic isomer (β-HCH) in considerable quantities, whereas for the more toxic γ-HCH, the bioconcentration was relatively lower (~1).

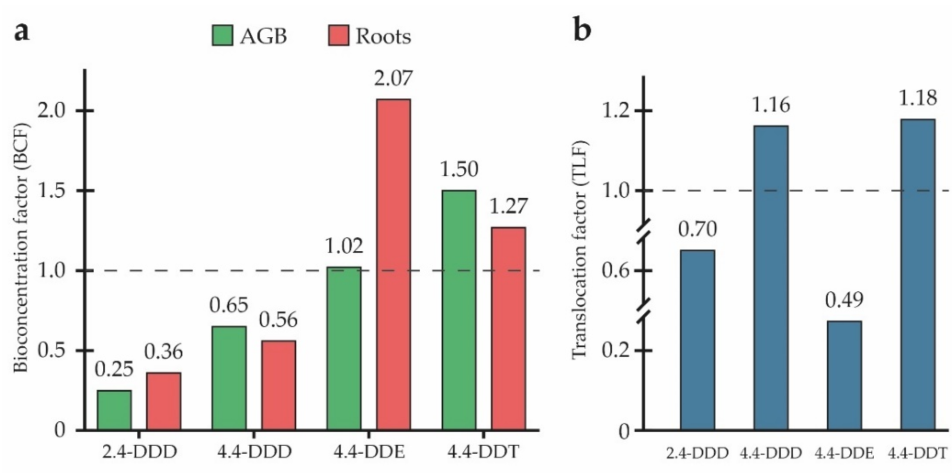

Figure 3 – *M. sinensis* phytoremediation potential concerning DDT & metabolites: a) BCF; b) TLF

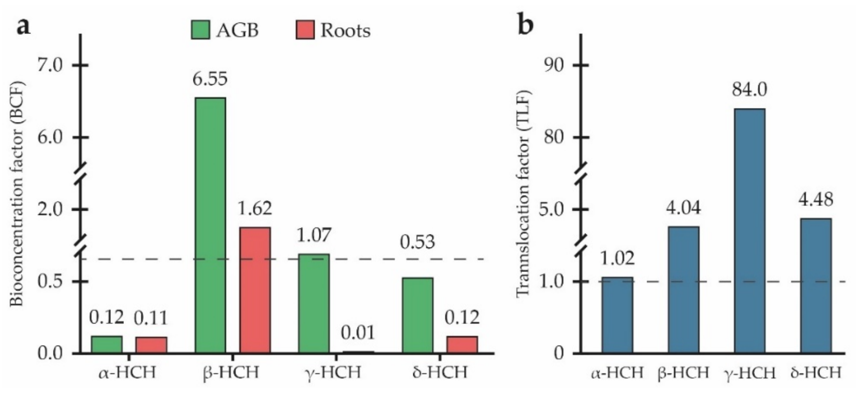

Figure 4 – *M. sinensis* phytoremediation potential concerning HCH isomers: a) BCF; b) TLF

Investigation of *M. sinensis* phytoremediation potential evaluating solely POP-pesticides concentrations data presents certain limitations. In general, POP-pesticides distribution in *M. sinensis* tissues correlates with their $log\ K_{ow}$ values: higher hydrophobicity ($log\ K_{ow}$) typically leads to reduced accumulation. However, this trend did not hold for two substances: 2.4-DDD and β-HCH.

Indeed, low concentrations of 2.4-DDD in the plant biomass could be attributed to its transient state during the anaerobic degradation of DDT, eventually forming 2,2-bis(4-chlorophenyl)-acetic acid [43]. Furthermore, the correlation anomaly for DDD might be due to its high octanol-air partition coefficient ($log\ K_{OA}$ = 10.1, compared to 9.82 for 4.4-DDT and 9.68 for 4.4-DDE). Organic compounds with low $log\ K_{ow}$ but high $log\ K_{OA}$ values could not typically accumulate in plant tissues [44]. Further, the peculiar behaviour of 2.4-DDD could be linked to its metabolically activated nature and potential breakdown into two metabolites: *o.p'*-DDA and *o.p'*-DDE [45].

To understand the behaviour of *β*-HCH, its $log\ K_{OA}$ value should be taken into account. This coefficient is crucial for understanding the dynamics of organic compounds between air and environmental matrices such as soil, vegetation, and aerosol particles. For *β*-HCH, the $log\ K_{OA}$ is equal to 8.1, while for γ-HCH, it is 9.7, which accounts for the lower accumulation of the latter. Moreover, contaminants with low $log\ K_{ow}$ and high $log\ K_{OA}$, as in the case of *β*-HCH, were reported to be unable to significant bioaccumulation [44].

Thus, the phytoremediation potential of *M. sinensis* in relation to POP-pesticides detected in research soil is summarized in Table 3.

Table 3 – Summary of *M. sinensis* phytoremediation potential in relation to studied POP-pesticides.

POP-pesticide	BCF >1			TLF > 1	Phytoremediation potential
	AGB	Roots	Mean		
DDT and metabolites					
2.4-DDD	–	–	–	–	No potential
4.4-DDD	–	–	–	+	No potential
4.4-DDE	+	+	+	–	Phytostabilization
4.4-DDT	+	+	+	+	Phytoextraction
	HCH isomers				
α-HCH	–	–	–	+	No potential
β-HCH	+	+	+	+	Phytoextraction
γ-HCH	+	–	–	+	Phytoextraction
δ-HCH	–	–	–	+	No potential

Conclusion

The investigation into the potential of *M. sinensis* as a tool for POP-pesticides-contaminated soils has yielded promising results. This novel phyto plant has exhibited robust growth, showcasing its resilience and adaptability in POP-pesticides-contaminated environments. Furthermore, our study has confirmed its ability to effectively accumulate the studied POP-pesticides. The process of POP-pesticide accumulation within *M. sinensis* was found to be influenced by several key factors, including pesticide hydrophobicity, molecular weight, and concentration in soil. These variables played a crucial role in determining the extent and efficiency of contaminant translocation within the plant. Importantly, our findings revealed that POP-pesticide accumulation mostly occurred uniformly across both aboveground biomass and roots.

Furthermore, *M. sinensis* has also demonstrated remarkable adaptability by employing distinct phytoremediation strategies depending on the specific POP-pesticides present in the contaminated soil. Our observations have revealed that *M. sinensis* exhibits phytoextraction capabilities concerning 4.4-DDT, β-HCH, and γ-HCH, achieving TLF of up to 84. Conversely, the plant displayed phytostabilization activity when encountering 4.4-DDE, a critical compound that marks the final degradation product of DDT, the primary POP-pesticide found in Kazakhstan. Given the hydrophobic nature of 4.4-DDE ($log\ K_{ow}$ of 6.51), *M. sinensis* effectively stabilizes this compound within its roots.

In conclusion, *M. sinensis* ability to apply distinct phytoremediation strategies depending on the specific contaminant present in soil positions it as an asset in addressing soil contamination while simultaneously offering the prospect of producing clean biomass for various applications. Further research on optimizing the efficacy of phytoremediation using *M. sinensis* for POP-pesticide contaminated soils is necessary to expand the range of POP-pesticides that *M. sinensis* can effectively manage through phytostabilization.

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