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Pumpkin and Its Extraction Capacities for Organochloride Pesticides in Kazakhstan

This study is aimed at the determination of pesticide-tolerant pumpkin species and related resources across the *Cucurbitae* spp. to be further used for phytoremediation of pesticide-contaminated soils in Almaty region of Kazakhstan. The data have shown particular species of the *Cucurbitae* grown on the soils contaminated by the pesticides which would be able to uptake DDT and HCH metabolites in amounts exceeding maximum allowable concentration almost 19 times.

Eleven food pumpkin and heirloom pumpkin varieties including few giant pumpkin varieties, *Cucurbita maxima* Duchesne ex Lam. have been introduced in the steppe zone of Almaty region. Hybrid fruits obtained from certain combinations have been shown to reach 30.0 x 20.0 cm in size. Kazakhstan researchers are looking forward a broad collaboration on pumpkin collection, pesticide neutralization, decomposition based on phytoextraction, phytostabilization and other techniques.

Key words: pumpkin, organochloride pesticide, phytoremediation, soil detoxication

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Тыква и ее экстрактивные свойства к органохлоридным пестицидам в Казахстане

Данное исследование посвящено определению пестицидоустойчивых сортов образцов и видов тыквенных, которые в перспективе будут применяться для загрязненных такими пестицидами почв Алматинской области. Приведены данные по специфическим сортам образцам *Cucurbitae*, выращенным на загрязненных почвах. Эти сорта образцы способны воспринимать ДДТ и ГХЦГ метаболиты в концентрациях, превышающих предельно-допустимые почти в 19 раз.

Одиннадцать сортов образцов пищевой и декоративной тыкв, включая сорта гигантской тыквы, *Cucurbita maxima* Duchesne ex Lam. были интродуцированы в степной зоне Алматинской области. Гибридные формы, полученные от определенных комбинаций, как было установлено, достигали размеров 30 x 20 см. Казахские исследователи готовы к широкому сотрудничеству по коллекции тыкв, а также по вопросам нейтрализации, разложения пестицидов на основе фитоэкстракции, фитостабилизации и других методов.

Ключевые слова: тыква, органохлоридные пестициды, фиторемедиация, почвенная детоксикация

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Introduction

The New World genus *Cucurbita* (pumpkins, yellow-flowered gourds and squashes) is composed of 12–14 species distributed from the U.S. to Argentina. At least five different species were domesticated before the European Contact, forming important sources of food in native American economies, and some of these species were among the earliest plants taken under cultivation and domesticated in the New World [1].

Soil remediation by implication of effective measures to technogenically polluted soils is one of ecological priorities for this country. Importance of cleaning techniques is obvious due to soil contamination with pesticides originated from the former storehouses of plant protection chemicals that had been in operation during the Soviet era. Soils surrounding such depositories, though currently out of operation, are polluted with DDT and HCH isomers in concentrations 78 times exceeding maximum allowable concentration (MAC, [2]). Similar outputs were obtained for 20 territories of the former pesticide depots in Akmola region [3]. These lands have appeared to become poisonous “hot spots” presenting a conspicuous environmental threat.

Natural recovery processes enhanced by microbial DDT degradation have been referred as being not much effective. The reason for that is a long-lasting period of half-disintegration and slow degradation by soil bacteria [4]. This may cause the urgencies of elaboration for alternative technologies to the recovery of polluted soil. Conventional technologies of soil purification for pesticide-containing lands are energy-consuming as demanding substantial investments. One of the strategies preventing environmental pollution is application of phytoremediation techniques. Major steps of soil recovery by using plants under contamination with organochloride pesticides are phytoextraction and phytostabilization [5] Choice of particular plants for this approach is determined by their ability to take up soil moisture to the surface at the rate of transpiration, then cleave pollutants by implication of internal enzymes and accumulate hazardous concomitants in biomass [6-8].

Plant's phytoextraction potential depends on pollutant's hydrophobicity. The degree of hydrophobicity ($\log K_{ow}$) is considered to be the major parameter in determining the effectiveness of concomitants' uptake in the plant. Toxic agents

including DDT and HCCH possessing $\log K_{ow}$ in the range of 3.5 and 8.3 are attributed to steady organic pesticides. In soil these agents occur to be bound by the process of chelating with inorganic and organic matters occurring thereby isolated by natural solid soil particles, what leads to their reduced bioavailability to flora [5, 9]. However, in 90-s it has been shown that the species *Cucurbita pepo* L. would be able to accumulate soil hydrophobic pollutants. Presence of dioxins and furans in leaves and fruits of this species after burning has witnessed in favors of DDT translocation in plant through the network composed of soil, root system and above-ground [10]. Subsequently, researchers from Yale University, USA holding the leading position in phytoremediation studies established that pumpkin would possess the highest phytoextraction potential enabling the accumulation of up to 1.5% of organochloride pesticides [11]. Ability of other plant species to extract organochloride pesticides from polluted soils has been reported, too. Particularly, transfer of insecticides, namely organochloride pesticides from soil has been recorded for tissues of lettuce (*Lactuca sativa*), alfalfa (*Medicago sativa*), cucumber (*Cucumis sativus*), rapeseed (*Brassica napus*), common bean (*Phaseolus vulgaris*), *Hanthium strumarium* and many other species as crops [12-15]. All these data have paved the way to consequent search of plant resources exhibiting phytoremediation features in respect of hydrophobic pollutants. Therefore, it sounds reasonable to study various representatives of *Cucurbita pepo* from original domestic and international germplasms on their ability of extracting DDT metabolites and HCH isomers from polluted soil.

Materials and methods

Pumpkin cultivars used in present study (cvs. “Monarch's Crown” (“Shapka Monomakha”), “Mantnaya”, “Vitamin” as heirloom pumpkins mentioned below have been chosen as productive local or introduced cultivars with so far unknown characters under pesticide treatment.

To develop phytoremediation techniques, seeds of *Cucurbita pepo* have been sown under greenhouse conditions into soil contaminated with pesticides. Used artificial contaminated soil from a solution of α -HCH, β -HCH, 4,4 DDE, 2,4 DDD, and 4,4 DDD. Average total pesticide in soil has reached 500 μg per 1 kg of soil. A control treatment utilized clean soil. Three replications were taken at each sampling. Residual concentrations of organochlo-

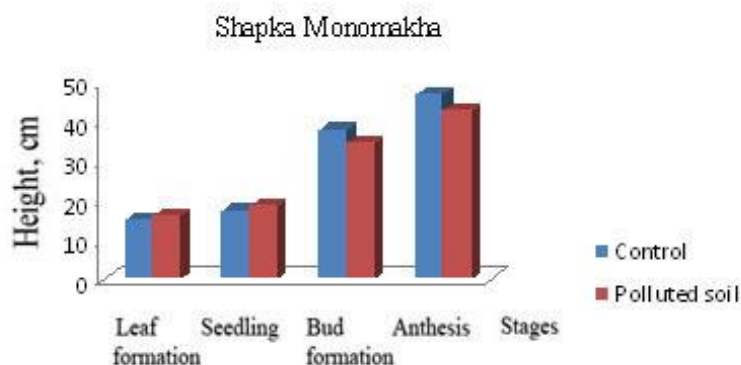


Figure 1 – Growth and development of *Cucurbita pepo*, cv. "Shapka Monomakha"

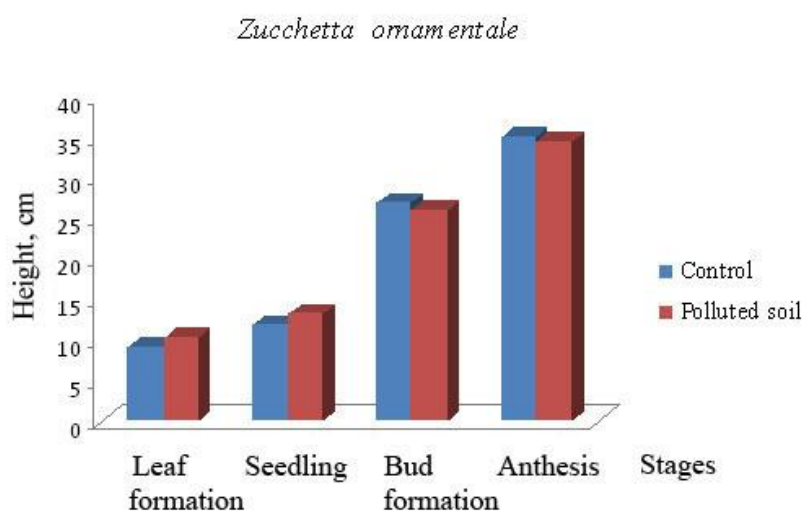


Figure 2 – Growth and development of *Cucurbita pepo*, *Zucchetto ornamentale*

Table 1 Phytoextraction potential of food and heirloom pumpkins grown on pesticide-polluted soil.

Pumpkin variety and origin	Vegetative organs	Pesticide concentration $\mu\text{g kg}^{-1}$	BCF *	TCF**
Domestic food pumpkin, Kazakhstan	Above-ground	15.60±1.70	12.50	0.04
	Root	371.80±2.10		
"Shapka Monomakha" ("Monarch's Crown"), Russia	Above-ground	29.50±6.0	1.64	3.20
	Root	9.20±1.30		
"Vitamin" Pumpkin, Russia-Kazakhstan	Above-ground	48.80±10.04	2.50	2.80
	Root	31.80±2.30		
"Zucchetto ornamentale", Italy	Above-ground	207.90±6.80	6.10	1.70
	Root	121.50±4.20		
"Griffe du Diable", France	Above-ground	207.90±60.80	0.35	1.80
	Root	12.20±0.80		

*BCF, pesticide bioconcentration factor
 **TCF, pesticide translocation factor

rine pesticides in soil and plant were determined using standard methods adopted by the United States Environmental Protection Agency using a gas chro-

matograph 6890 (Agilent, USA) equipped with an electron capture detector and a capillary column using EPA methods 8081 and 8250A (USEPA 2007).

Concentration of SDDT and SHCH in soils was calculated from SDDT standards run with each batch of samples gas chromatography. Results obtained for 4,4'-DDD, 4,4'-DDT, 4,4'-DDE, 2,4'-DDD, α -HCH, β -HCH and γ -HCH were expressed as $\mu\text{g kg}^{-1}$ of pesticides per gram wet weight of soil/ plant tissue.

As estimated criteria of accumulative ability of plants following parameters were used: the residual amount of pesticides in above-ground organs and roots of plants, in $\mu\text{g kg}^{-1}$; factor translocation pesticides (was calculated as [SDDT] or [SHCH] shoot/[SDDT] or [SHCH] root to reflect pollutant's amount transferred to shoots with respect to its amount in roots; bioconcentration factor (was calculated as the residual amount of pesticides in tissue of plants/soil before experiment). The statistical processing of our data was carried out using MS EXCEL software.

Results and discussion

As shown in figures 1 and 2, to reveal changes of plant physiological processes under conditions of experimental pollution, phenology observations have been fulfilled. Despite 5 times higher concentrations if compared with soil MAC, plants have been shown to undergo a complete life cycle. Moreover, polluted soil has induced accelerated plant developmental stages reducing the period of ontogenesis. Cv. "Mantnaya", when vegetated on unpolluted, clean soil, has been determined to reach anthesis on 64-th day, whereas on pesticide-contaminated soil it has flowered on 55-th day of development. Similar alterations have been noticed for cv. "Monarch's Crown" ("Shapka Monomakha"). However, under these conditions pesticides have been stated to depict no significant effect on plant height for food and heirloom pumpkins.

This parameter has been detected to remain at the same level or not more than 9% taller than the height of plants grown on polluted soil.

During determination of the residual amount of pesticides in above-ground organs and roots of representatives of Cucurbita spp. it has been confirmed that they would possess ability to extract pesticides from polluted soil. At growing of different varieties of pumpkin until the stage of flowering on soils polluted with pesticides, pesticide concentration in plant tissue exceeds MAC up to 19 times. MAC for plant tissue in Kazakhstan is $20 \mu\text{g kg}^{-1}$. (Table 1).

Interestingly, domestic food pumpkin and Italian heirloom species *Zucchetta ornamentalis* have depicted high accumulation ability. Food pumpkin varieties have shown the uptake of $387.4 \mu\text{g kg}^{-1}$ of pesticides in vegetative organs, where as *Z. ornamentalis* ($239.7 \mu\text{g kg}^{-1}$). Under these conditions *Z. ornamentalis* has been detected to sample $207.9 \pm 6.8 \mu\text{g kg}^{-1}$ in above-ground part, and $121.55 \pm 4.2 \mu\text{g kg}^{-1}$ in roots. The data witness in favour of plant's ability to transfer pesticides from soil through roots to above-ground organs. Representatives of the Cucurbitae have been established to accumulate in plant organs predominantly 4,4' DDE, 4,4' DDT and β -HCH metabolites. Besides these isomers, slight accumulation of other metabolites such as 2,4' DDD, α -HCH and γ -HCH has been identified. Meanwhile, according to regulations of the Republic of Kazakhstan, their presence in soil and crops is completely inadmissible. Ability of various plants to accumulate pesticides in tissues is anticipated to be dependent on the species characteristics, anatomic and morphological structure and peculiarities of metabolism.

Ability of the pollutant to migrate from root to above-ground organs and related translocation factor have been investigated. Root system is major target involved in pesticide accumulation. However, some varieties have manifested their ability to transfer pesticides from roots to the above-ground tissues. For example, translocation factor for *Z. ornamentalis* has made up 1.7. Similar data have been obtained for other pumpkin species and varieties. High BCF index has been shown for cv. "Shapka Monomakha" (BCF=3.2).

Based on these data we have assumed that the Cucurbitae plants are able to uptake pesticides and transfer them from roots to above-ground organs thereby facilitating soil purification. By such ability the Cucurbitae spp. are attributed to the group of effective plant resources able to acropetally transfer organochloride pesticides. Current data have confirmed earlier observations that Cucurbita pepo would possess phytoextraction potential [11].

Thus, representatives of the Cucurbitae (domestic food pumpkin and *Z. ornamentalis*) have demonstrated pesticide-accumulating activity 19 times exceeding MAC and may be listed among most effective plants enabling soil recovery under its contamination with hydrophobic pesticides.

Eleven food pumpkin and heirloom pumpkin varieties have been introduced in the steppe zone of Almaty Region in 2012 including few giant pumpkins, *C. maxima*. Next year this number has been twice increased.

Countries with traditional areas for vegetables cropping, breeding and research [16] are in the focus of ongoing phytoremediation research in Kazakhstan in order to overview new forms of collaboration on this specific as acute environmental issue impeding sustainable development world-wide.

Conclusion

Irrespective of hugely exceeding concentrations with regard to upper soil MAC pumpkin plants have been shown to develop in accordance with a complete life cycle. Strikingly, soil contamination with pesticides has brought speedy plant development in shorter period of ontogenesis decreasing by 9-10 days. Pesticides under such conditions have been indicated to reveal no visible effect on plant height among food and heirloom pumpkins. Domestic food

pumpkin cultivars and Italian heirloom species *Zucchetta orna-mentale* have shown a high pesticide accumulation ability with the uptake reaching 390 and 210 $\mu\text{g kg}^{-1}$ of pesticides in above-ground part, respectively. The data reflect the ability of plants to transfer pesticides from soil through roots to above-ground organs. 4,4'-DDE, 4,4'-DDT and β -HCH metabolites have been determined to occur dominating agents stored in plant organs. Besides, slight accumulation of other isomers including 2,4'-DDD, α -HCH and γ -HCH has been detected. Plant ability to depositing pesticides in tissues is believed to depend on the species characteristics, anatomic and morphological structure and peculiarities of metabolism. Kazakhstan is looking forward a wider international collaboration for the development of pumpkins collection as effective resolution of phytoremediational issues for pesticide neutralization, decomposition based on phytoextraction, phytostabilization and other techniques.

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