Pumpkin as Extraction Tool for Organochloride Pesticides in Kazakhstan

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Abstract

Current strategy is aimed at the identification of pesticide-resistant plant resources among the species of the *Cucurbitae* which may be used for phytoremediation of pesticide-contaminated soil in Almaty region of Kazakhstan. The data have shown that the species of the *Cucurbitae* grown on soils polluted with pesticides would be able to accumulate metabolites of DDT (p,p'-dichlorodiphenyltrichloroethane) and HCH (hexachlorocyclohexane) in quantities exceeding maximum allowable concentration (MAC) almost 19 times. Eleven food pumpkin and heirloom pumpkin varieties including few giant pumpkin varieties, *Cucurbita maxima* have been introduced in the steppe zone of Almaty region in 2012. Hybrid fruits obtained from certain combinations have been shown to reach 30.0 x 20.0 cm in size.

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

Abbreviations: BCF , pesticide bioconcentration factor; DTT, dichlorodiphenyltrichloroethane;HCH, hexachlorocyclohexane; MAC, maximum allowable concentration; TCF, pesticide translocation factor

Introduction

Design of effective approaches for soil remediation to technogenically contaminated soils is one of principal ecological objectives in the Republic of Kazakhstan. Significance of this issue and related cleaning techniques is clear due to the soil contamination with pesticides, especially considering former depositories of plant protection chemicals which had been in operation over the Soviet period. According to the data the soil surrounding such chemical storehouses, which are presently out of operation, is polluted by DDT and HCH isomers in concentrations 78 times exceeding maximum allowable concentration (1). Similar outputs have been obtained under the survey on 20 territories of the former pesticide depots in Akmola region (2). 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 (3). 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 (4). 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 (5-7).

Plant's phytoextration 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 (4, 8). 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 (9). 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 (10). 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 letuce (*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

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 organochlorine pesticides in soil and plant were determined using standard methods adopted by the United States Environmental Protection Agency using a gas chromatograph 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 obtains for 4,4'-DDD, 4,4'-DDT, 4,4'-DDE, 2,4'-DDD, α-HCH, β-HCH and γ-HCH were expressed as μg 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 μg 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

To reveal changes of plant physiological processes under conditions of experimental pollution, phenology observations have been fulfilled. Despite 5 times exceeding concentrations when compared to upper maximum allowable concentration in soil, plants have been shown to undergo a complete life cycle. Moreover, soil pollution with pesticides has led to accelerated plant developmental stages and reduction of time for ontogenesis. For example, cv. "Mantnaya" food pumpkin, when harvested on uncontaminated, clean soil, has been shown to reach a thesis on 64-th day, whereas when grown on pesticide-contaminated soil – on 55-th day of development. Similar alterations have been registered for cv. "Shapka Monomakha" ("Monarch's Crown"). However, under these conditions pesticides have been stated to depict no significant effect on plant height for food and heirloom pumpkins (Fig. 1 and Fig. 2).

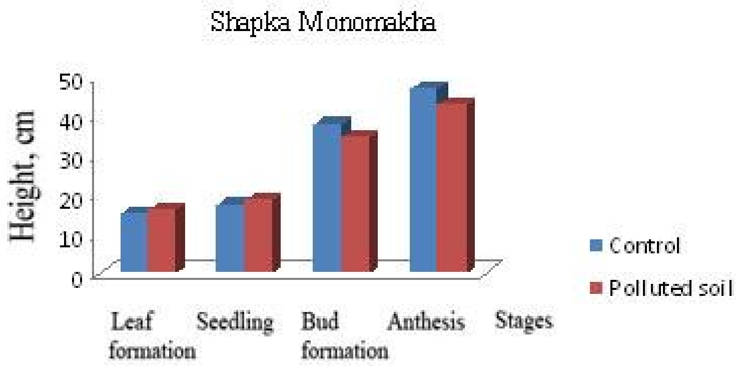

Fig.1 Growth and development of *Cucurbita pepo,* cv. "Shapka Monomakha"

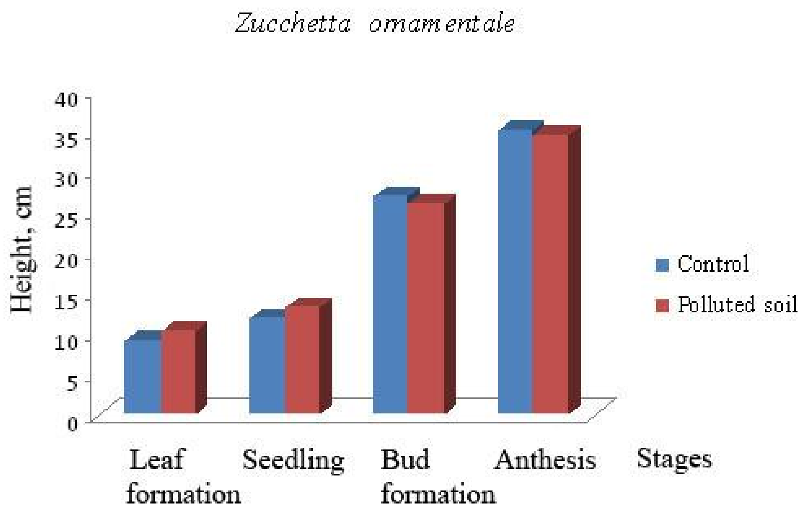

Fig.2 Growth and development of *Cucurbita pepo, Zucchetta ornamentale*

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 μg kg^{-1}. (Table 1).

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

Pumpkin variety and origin	**Vegetative organs**	**Pesticide concentration μg 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		
"Zucchetta 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

Interestingly, domestic food pumpkin and Italian heirloom species *Zucchetta ornamentale* have depicted high accumulation ability. Food pumpkin varieties have shown the uptake of 387.4 μg $kg^{-1}$of pesticides in vegetative organs, where as Z. *ornamentale* (239.7 μg kg^{-1}). Under these conditions *Z. ornamentale* has been detected to sample 207.9±6.8 μg kg^{-1} in above-ground part, and 121.55±4.2 μg 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 ß-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.

One of the requirements to phytoremediation use is ability to enable pollutant's migration from root to above-ground organs of plants. For definition of intensity of the pesticide migration in "soil – root – above-ground organs" system the translocation factor has been applied. Root system is major target involved in pesticide accumulation. Most pesticides are accumulated in the root system; however, out of the species under investigation some species demonstrated ability to transfer pesticides from roots to the above-ground tissues. For example, translocation factor for Z. *ornamentale* has occurred to make up 1.7. Similar results have been obtained for other pumpkin species and varieties subject to introduction. High index of bioconcentration has been shown for cv. "Shapka Monomakha" (BCF 3.2).

Based on these data it is possible to assume that plants belonging to the *Cucurbitae* are able to accumulate pesticides in vegetative organs and transfer pesticides from roots to above-ground organs thereby facilitating soil detoxication from pesticides.

By the ability to the foster pesticide migration in "soil-plant" system representatives of the *Cucurbitae* are attributed to the group of effective plant resources able to transfer organochloride pesticides acropetally from roots to upper part of plants. Our data have confirmed this observation advanced earlier for stating that plants of *Cucurbita pepo* would possess phytoextraction potential (10).

Thus, representatives of the *Cucurbitae* (domestic food pumpkin and *Z. ornamentale*) 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 pumkin varieties have been introduced in the steppe zone of Almaty Region in 2012 including few giant pumpkins, *C. maxima*. Hybrids obtained from some combinations to be further examined are listed in Table 2.

Table 2 Size of food and giant pumpkin hybrids

Description	Size, cm
100 pound pumpkin x Tolstushka	28.5 x 18.0
Volzhskaya seraya x Lechebnaya	28.0 x 21.0
Lechebnaya x 100 pound pumpkin	25.0 x 22.0
Tsukat x Tolstushka	29.0 x 15.0

Balkan countries as traditional area of 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 worldwide.

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