Abstract
Present study is targeted to the determination of pesticide-resistant plant resources based on available species of the Cucurbitae which may be used for phytoremediation of pesticide-polluted soil in Almaty region of Kazakhstan.

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

Introduction
Effective soil remediation approaches to technogenically polluted soils is one of principal ecological objectives in the Republic of Kazakhstan. This issue and related cleaning techniques are essential under current soil contamination with pesticides, especially regarding the former depositories of plant protection chemicals which had been then in full operation over the Soviet period. According to the data, soils surrounding such huge storehouses, which are out of operation at present, is polluted with DDT and HCH isomers in amounts exceeding maximum allowable concentration (MAC) more than 78 times [1]. Similar results have been shown due to the survey on 20 territories of the former pesticide depots in Akmola region [2]. These lands have occured to become hazardous environmental “hot spots”.

Natural soil recovery provided for by microbial DDT degradation has been referred to as being not much effective. Long-lasting period of half-disintegration and slow degradation by soil bacteria were pointed [3] as the reason. This might has caused the necessity of elaborating alternative technologies for the recovery of polluted soil. Conventional technologies of soil purification on pesticide-affected lands are regarded as energy-consuming or demanding substantial investments. One of the strategies preventing poisonous soil pollution is application of phytoremediation techniques. Major steps of soil recovery by plants in result of soil contamination with organochloride pesticides are phytoextraction and phytostabilization [4]. Choice of plant species for this approach is determined by their ability to transfer soil moisture at the rate of transpiration and then cleave diluted concominants by cell enzymes to accumulate hazardous pollutants in biomass [5-7].

Plant’s phytoextraction potential depends on pollutant’s hydrophobicity. The extent of hydrophobicity (log Kow) is attributed to major parameter in respect of the effectiveness of pollutant’s uptake in the plant. DDT, HCH and other toxic agents possessing log Kow in the range of 3.5 and 8.3 are referred to steady organic pesticides. In soil these agents occur to be bound owing to chelating with inorganic and organic matters appearing thereby isolated inside natural solid soil particles, what reduces their bioavailability to flora [4, 8]. However, in 90-s it has been shown that pumpkin, 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 confirmed DDT translocation in the seedling via specific network including soil, root system and above-ground [9]. Subsequently, researchers from Yale University, USA as the world leaders of phytoremediation studies have shown that pumpkin would possess the highest phytoextraction potential due to the sampling of almost 1.5% of organochloride pesticides [10]. Ability of other plant species to extracting organochloride pesticides from polluted soils has been reported, too. Particularly, transfer of insecticides, namely organochloride pesticides from
soil has been registered 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 [11-15]. All these data have led to subsequent extending research on plant resources displaying phytoremediation ability to hydrophobic contaminants. Hence, enrichment of pumpkin’s germplasm in Kazakhstan and the study on various representatives of *Cucurbita pepo* from original domestic and international germplasms on their ability of extracting DDT metabolites and HCH isomers from polluted soil are crucial issues for this country indeed.

**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 obtained 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 mg 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 understand alterations of plant physiological processes under experimental pollution, phenology observations have been undertaken. Despite 5 times exceeding concentrations comparing to MAC n soil, plants have been shown to undergo a complete life cycle. Moreover, soil pollution with pesticides has caused accelerated plant developmental stages and reduction for the period of ontogenesis. For instance, cv. “Mantnaya” food pumpkin harvested on clean, unpolluted soil, has been shown to reach a thesis on 64-th day, whereas the same variety grown on pesticide-contaminated soil – on 55-th day of development. Similar changes have been manifested 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).

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**Fig. 1** – Growth and development of *Cucurbita pepo*, cv. “Shapka Monomakha”
This parameter has been detected retaining at the same level or occurring only 9% taller than plant height in seedling grown on polluted soil.

Assessment of residual quantities of pesticides in above-ground organs and roots of *Cucurbita spp.* has depicted that plants would possess ability to extract pesticides from polluted soil. At growing of different pumpkin varieties up to the stage of flowering on soils polluted with pesticides, pesticide concentration in plant tissues has exceeded MAC up to 19 times.

Meanwhile, MAC for plant tissues in Kazakhstan is 20 mg kg\(^{-1}\) (Table 1).

It is worth to note that domestic food pumpkin and Italian heirloom species *Zucchetta ornamental* have shown a high “outpumping” ability. Food pumpkin varieties have shown the uptake at the level of 387.4 mg kg\(^{-1}\) of pesticides in vegetative organs, where as *Z. ornamental* 239.7 mg kg\(^{-1}\). Under these conditions *Z. ornamental* has been detected to sample 207.9±6.8 mg kg\(^{-1}\) in

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

<table>
<thead>
<tr>
<th>Pumpkin variety and origin</th>
<th>Vegetative organs</th>
<th>Pesticide concentration, mg kg(^{-1})</th>
<th>BCF *</th>
<th>TCF**</th>
</tr>
</thead>
<tbody>
<tr>
<td>Domestic food pumpkin, Kazakhstan</td>
<td>Above-ground</td>
<td>15.60±1.70</td>
<td>12.50</td>
<td>0.04</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>371.80±2.10</td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Shapka Monomakha” (&quot;Monarch’s Crown&quot;), Russia</td>
<td>Above-ground</td>
<td>29.50±6.0</td>
<td>1.64</td>
<td>3.20</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>9.20±1.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Vitamin” pumpkin, Russia-Kazakhstan</td>
<td>Above-ground</td>
<td>48.80±10.04</td>
<td>2.50</td>
<td>2.80</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>31.80±2.30</td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Zucchetta ornamental”, Italy</td>
<td>Above-ground</td>
<td>207.90±6.80</td>
<td>6.10</td>
<td>1.70</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>121.50±4.20</td>
<td></td>
<td></td>
</tr>
<tr>
<td>“Griffe du Diable”, France</td>
<td>Above-ground</td>
<td>207.90±60.80</td>
<td>0.35</td>
<td>1.80</td>
</tr>
<tr>
<td></td>
<td>Root</td>
<td>12.20±0.80</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Note: *BCF*, pesticide bioconcentration factor; **TCF**, pesticide translocation factor.

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Fig.2 – Growth and development of *Cucurbita pepo, Zucchetta ornamental*
above-ground organs, and 121.55±4.2 mg 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 this, slight accumulation of other metabolites, and namely 2.4’ DDD, a-HCH and g-HCH, has been identified. However, according to regulations of the Republic of Kazakhstan, presence of these pollutants in soil and crops is not allowed. 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” network the translocation factor has been implied.

Root system is a major target involved in pesticide accumulation. Most pesticides are accumulated in roots, though some species demonstrate their ability to transfer pesticides from roots to the above-ground tissues. For instance, translocation factor (TCF) for *Z. ornamentale* has occured 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 of the *Cucurbitae* are able to accumulate pesticides in roots or 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” network the *Cucurbitae* are attributed to the group of effective plant resources able to transfer organochloride pesticides acropetally from roots to the above-ground. Our data have confirmed this observation put forward earlier to prove 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 pumpkin 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

<table>
<thead>
<tr>
<th>Description</th>
<th>Size, cm</th>
</tr>
</thead>
<tbody>
<tr>
<td>100 pound pumpkin x Tolstushka</td>
<td>28.5 x 18.0</td>
</tr>
<tr>
<td>Volzhskaya seraya x Lechebnaya</td>
<td>28.0 x 21.0</td>
</tr>
<tr>
<td>Lechebnaya x 100 pound pumpkin</td>
<td>25.0 x 22.0</td>
</tr>
<tr>
<td>Tsukat x Tolstushka</td>
<td>29.0 x 15.0</td>
</tr>
</tbody>
</table>

It has been also shown that size of heirloom pumpkin (*Z. ornamentale*) can be increased by 20% and more by the application of a new organic fertilizers offered by KazNU chemists (Fig. 3 and Prof. M. Aldabergenov’s pers. com).

Vegetables cropping, breeding and research [16] are in the focus of ongoing phytoremediation studies here, in Kazakhstan, in order to design new collaboration ties on this particular as acute environmental issue impeding sustainable development worldwide.
Fig. 3 – Growth and development of Zucchetta ornamentale. Upper row – control growth, lower row – application of experimental fertilizer.

References


