

# The Problem of Obsolete Pesticides Pollution for the Kazakhstan Environment and Soil Remediation by Wild Plants

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## ABSTRACT

The development of remediation technologies for soils polluted by pesticides is an important problem in Kazakhstan. Phytoremediation is one of the potential methods for reducing risk from these pesticides. We have investigated the territory surrounding former pesticide storehouses of a former plant protection system for collective farms of the former USSR. Now, the storage buildings have been destroyed, and there is no security or fencing around these sites. At the sites we studied, organochlorine residues found in soil included metabolites of DDT (dichlorodiphenyltrichloroethane) and isomers of HCH (hexachlorocyclohexane). Soil samples were collected from each pesticide storehouse site to examine migration and expansion of pollution. Sites with soil contamination observed in excess of maximum acceptable concentrations (MACs) for the Republic of Kazakhstan were called hot points. Twenty-four of the storehouse sites showed soil concentrations in excess of MACs. The MAC for Kazakhstan soil is 100 µg/kg for DDT metabolites (4,4 DDT; 4,4 DDE) and HCH isomers (βHCH; γHCH). Three compounds we analyzed (2,4 DDD, 4,4 DDD, and αHCH) did not have a MAC for Kazakhstan. Genetic diversity in populations of wild and weedy species that colonize pesticide-contaminated soil provides a source of plant species tolerant to these conditions. The strategy was to identify pesticide-tolerant plant genotypes which can be used for phytoremediation of pesticide-contaminated soil in the Almaty oblasts of Kazakhstan. The results have shown that colonizing plant species growing on soils polluted by pesticides possess the ability to accumulate organochlorine pesticide residues in plant tissue including roots, stems, leaves and fruit and to reduce pesticide concentrations in soil.

**Keywords:** accumulation, dichlorodiphenyltrichloroethane, hexachlorocyclohexane, isomers, metabolites, phytoremediation

## INTRODUCTION

Kazakhstan became independent from the former Soviet Union since 1991. However, many of the impending environmental problems were not anticipated. Within five years of independence, pesticide storage storehouses that used to be managed by the official plant protection service of the former Soviet Union were destroyed, leaving obsolete pesticides and their containers unattended and open to the environment (Nazhmetdinova 2001). Most of the bulk obsolete pesticides have been moved to other storage areas, taken by citizens for individual use, resold, or released into the surrounding environment with no indication of their potential danger to local residents. Much of the obsolete pesticides that were resold were first repackaged in unlabeled or mislabeled containers. People living around the warehouse sites often use the land for pasture, kitchen gardens, and play areas for children and as a source of construction materials. Pollution of soil and water by obsolete pesticides is a serious ecological problem. Many of these former warehouses have become hot points of contamination and represent a serious ecological danger.

Large-scale and expensive remediation technologies that may be effective for pesticide-contaminated soil and water are likely to be unacceptable in Kazakhstan due to limited finances and resources. Innovative natural remediation technologies like phytoremediation are promising if they can be shown to address cleanup requirements and can be effectively managed at an acceptable cost. Phytotechnology uses vegetation to accumulate, degrade, or stabilize environmental contaminants (Perkovich *et al.* 1995; Cunningham *et al.* 1996; White 2002; Nzengung and Jeffers 2001; Davis *et al.* 2002; Prasad 2003; Tsao 2003; Huang *et al.* 2007; Doty 2008; Dowling and Doty 2009).

In this study, pesticide analysis was limited to the organochlorine pesticides (OPs) DDT (p,p'-dichlorodiphenyltrichloroethane) and HCH (hexachlorocyclohexane), along with their associated metabolites and isomers: 2,4 DDD (p,p'-dichlorodiphenyl dichloroethane); 4,4 DDD; 4,4 DDT; 4,4 DDE (p,p'-dichlorodiphenyldichloroethylene); αHCH; βHCH; and γHCH). While these pesticides represent only a subset of all obsolete pesticides, they are important due to their status as persistent organic pollutants and as compounds that represent a much larger problem.

The purpose of this paper was to develop feasible methods to reduce ecological and human health risk at obsolete pesticide sites using phytotechnologies.

To investigate the potential use of phytoremediation, we delineated the following three tasks:

Task 1: Inventory former obsolete pesticide warehouses to document obsolete pesticide stockpiles and to characterize levels of soil contamination.

Task 2: Identify pesticide-tolerant plant species using surveys of plant community structure at selected "hot points".

Task 3: Study the fate and transport of pesticides in soil and plants in the greenhouse using soil collected from 'hot points'.

## MATERIALS AND METHODS

### Inventory former obsolete pesticide warehouses to document obsolete pesticide stockpiles and to characterize levels of soil contamination

We surveyed obsolete pesticide storehouses in 10 of 14 districts in Almaty oblast or prefecture. In each district, the Department of Plant Protection of the Ministry of Agriculture was contacted to

obtain locations of former pesticide storehouses and permission to access the sites. Local government authorities were contacted to receive further information on locations and permission to survey and sample each site. In this paper, we refer to the former storehouse sites where we have observed pesticide contamination as “hot points.”

The inventory included descriptions of conditions of the storehouse structures; estimation of bulk obsolete pesticide stockpiles and pesticide containers, inspection of storehouses and surrounding areas for pesticide contamination, assessment of vegetation growing at the sites, and public outreach. An inventory worksheet was developed to provide a systematic description of each location.

Our study focused on the analysis of OPs as a marker of field contamination. We took 800 soil samples to determine residual concentrations of OPs. Three replications were taken at each sampling.

Residual concentrations of OPs in soil were determined using standard methods adopted by the United States Environmental Protection Agency (US EPA) using a gas chromatograph (HP6890, Series GC System Hewlett Packard) equipped with an electron capture detector and a capillary column using EPA method 8081. Samples soils were extracted in a Soxhlet apparatus for 16 hrs with 300 mL methylene chloride. The extract was concentrated by a Snyder device to 1 mL and the solvent exchanged to hexane. The extract was applied to a Florisil column and diluted with hexane to 10 mL. A 3-5 mL fraction was transferred to a vial for analysis by gas chromatography to determine the composition and quantity of SDDT and SHCH in soils. The concentration of SDDT and SHCH in soils was calculated from SDDT standards (purchased from Chem Service “Altey” Almaty, Kazakhstan) run with each batch of samples gas chromatography. Results obtained for 4,4DDD, 4,4DDT, 4,4 DDE, 2,4DDD,  $\alpha$ HCH,  $\beta$ HCH and  $\gamma$ HCH were expressed as  $\mu\text{g}/\text{kg}$  of pesticides/g dry weight of soil (USEPA 2007).

### Identify pesticide-tolerant plant species using surveys of plant community structure at selected “hot points”

Greenhouse and field experimental works were performed on sites of Karasajsk District. Three former warehouse sites were included to our initial study. The first point (Point 1) is located 15 km from Almaty with an area of 80 m<sup>2</sup>. At Point 1, the destroyed foundation of the warehouse can be seen and local residents reside alongside the old warehouse. The second point (Point 2) is located 50 km away from Almaty with an area of 60 m<sup>2</sup>. At Point 2, the destroyed foundation can also be seen along with grazing cattle. White pesticide residuals lying on top of the soil and in the plants can also be seen and during certain times of the year can be smelled. The third point (Point 3) is located 36 km from Almaty. Point 3 is a concrete and asphalt platform with a total area of 100 m<sup>2</sup>. Point 3 differs from Point 1 and 2 in that there were remnants of old pesticide containers found at this site.

We studied plant community structure in areas surrounding each hot point to describe botanical diversity, to identify pesticide-tolerant plant species that may be useful for phytoremediation, and to understand the mechanisms of detoxification of soil by plants. The distribution and taxonomic identification of plant species used the Tahtadjan technique (1987) taking into account main morphological attributes and structural parameters of plant communities including: aspect, specific structure, area cover, plant distribution, abundance, plant community adaptations, frequency, phenological stage of development and vigor of each separate plant species.

### Study the fate and transport of pesticides in soil and plants in the greenhouse using soil collected from hot points

In a greenhouse pot study 13 of 17 pesticide-tolerant species were used to study the fate and transport of pesticides in the soil and plant system. All pots were in triplicate. The experiment utilized soil from two former warehouse sites (hot points 1, 2 and 3) to estimate the accumulative ability of plants that had naturally colonized an obsolete pesticide site.

Plant tissues (10 plants from each plot) were sampled at the

time of flowering to estimate plant biomass production and content of HCH isomers and DDT metabolites in root and above-ground plant tissue. Residual concentrations of chlororganic pesticides in plant tissue were determined using standard methods adopted by the US EPA using a gas chromatograph (HP6890) equipped with an electron capture detector and a capillary column using EPA method 8250A. Samples plants were extracted in a Soxhlet apparatus for 4-6 hrs using 250 mL of methylene chloride. The extract was concentrated by a Kuderna-Danizh apparatus for 15-20 min to 1 mL and the solvent was exchanged to hexane. The extract was applied to a Florisil column and was flushed through with 10 mL hexane and then concentrated down to 0.5 mL with nitrogen gas. A sub-sample was transferred to a vial for analysis by gas chromatography to determine the composition and quantity of SDDT and SHCH in the plants. Results obtained for OPs were expressed as  $\mu\text{g}/\text{kg}$  of pesticides/g dry weight of plant (USEPA 1994). Histological methods were used to confirm the localization OPs in plant tissues. In the period of plant flowering stems, leaves and roots were cut into small pieces (15-20 mm) in the middle part of each organ. Materials were fixed in 70°C ethanol. Anatomical transverse sections of stems, leaves and roots were carried out by using a freezing microtome (TOS-2, Russia). 15 sections of each organ of plant species per site were sampled. All transverse sections were examined with an MBI-6 microscope fixed with a Zorki camera (Prodina 1969; Esau 1980).

### Statistical analysis of data

As estimated criteria of accumulative ability of plants following parameters were used: a) The residual amount of pesticides in above-ground and root of plants, in  $\mu\text{g}/\text{kg}$ ; b) Accumulation of pesticides in tissue of plants (the residual amount of pesticides in tissue of plants multiplied on dry weight), in  $\mu\text{g}$ ; c) Translocation factor (were calculated as [SDDT] or [SHCH] shoot/[SDDT] or [SHCH] root) to reflect the amount of contaminant that is transported to the shoots relative to the amount that is in the roots (Zeeb *et al.* 2003).

The statistical processing of data was carried out using MS EXCEL.

## RESULTS AND DISCUSSION

### To monitor pollution centers by obsolete pesticides for minimization of ecological risk in Kazakhstan

In the field we located 10 areas of Almaty oblast with 64 former warehouse sites. There were 352 tons of obsolete pesticides of different classes: triazin, phosphororganic, organochlorine, phthororganic, tiocarbamate, 144 metal iron containers and 106 cellophane and paper packages. Obsolete pesticides accounted for 315 tons, i.e. 89.6% (Figs. 1-4).

OPs were present as hazardous substances in soil around of 24 former warehouses where their concentration exceeded the MAC value by tens to hundreds of times. The basic pollutants were isomers of  $\alpha$  HCH,  $\beta$  HCH and metabolites of 4,4 DDE and 4,4 DDT. For example, in the Eskeldin District (Aldabergenov Village), the concentration of 4,4 DDT exceeds the MAC value by 19 times ( $1955 \pm 69 \mu\text{g}/\text{kg}$ ), 4,4 DDE by 28 times ( $2867 \pm 68 \mu\text{g}/\text{kg}$ ), and  $\beta$  HCH by 17 times ( $1731 \pm 117 \mu\text{g}/\text{kg}$ ). In the Talgar District (Kyzyl-Gairar District) the level of  $\alpha$ HCH is  $1239 \pm 136 \mu\text{g}/\text{kg}$ , 2,4DDD =  $398 \pm 8 \mu\text{g}/\text{kg}$ , and 4,4DDD =  $1899 \pm 42 \mu\text{g}/\text{kg}$ . These data show the ecological danger of these areas and also represent a significant potential risk to nearby populated areas. In Balkhash, Uigur and Ilijsk Districts an insignificant amount of HCH isomers exists. Isomers of HCH are highly toxic preparations, and cause mutagenic effects (Medbed 1977; Au *et al.* 1999; James *et al.* 2002).

Residual metabolites of OPs we observed in soil do not depend on the presence or absence of bulk, obsolete pesticide stockpiles at the storehouses. For example, in the village of Belbulak in Karasajsk District, 500 kg of unidentified white powders were observed in the open air. The observed soil concentration of 4,4 DDT exceeded MAC by



**Fig. 1** Residues of obsolete pesticides on site the former blasted storehouse pesticides located in Ulisk district of Almaty oblast. **Fig. 2** Scattered non-identified residues of obsolete pesticides and containers on site of former blasted storehouse pesticides located in Dzhambul district of Almaty oblast. **Fig. 3** Scattered non-identified residues of obsolete pesticides and containers on site of former blasted storehouse pesticides located in Uigur district of Almaty oblast. **Fig. 4** Scattered non-identified residues of obsolete pesticides on site of former blasted storehouse pesticides located in Karasajsk district of Almaty oblast. **Fig. 5** Species *Artemisia annua* growing on site of former blasted storehouse pesticides located in Karasajsk district of Almaty oblast (h – 3 m). **Fig. 6** Species *Erigeron canadensis* growing on site of former blasted storehouse pesticides located in Karasajsk district of Almaty oblast (h – 7-9 m).

16-fold ( $1670 \pm 66 \mu\text{g/kg}$ ) and 4,4 DDE exceeded MAC by 8-fold ( $852 \pm 18 \mu\text{g/kg}$ ). In the village of Kyzyl-Gairar in Talgar District, no pesticide stockpiles were observed but soil concentrations of 4,4 DDT exceeded MAC by 65-fold ( $6584 \pm 207 \mu\text{g/kg}$ ) and 4,4 DDE by 20-fold ( $2097 \pm 54 \mu\text{g/kg}$ ).

Control soil samples were selected at least 800 m from each hot point (Karasajsk District). Control samples contained some DDT and  $\alpha\text{HCH}$  metabolites. DDT metabolites primarily included 4,4DDE and 4,4DDT, but these did not exceed MACs.

Our results showed that the former pesticide storehouses were dangerous centers of environmental contamination. Soil around the former storehouses of pesticides has been polluted by OPs. Data showing the ecological danger of these areas also represents a significant potential risk to nearby populated areas. It is necessary to take urgent measures to liquidate, at first, all obsolete pesticides and their containers (burial place) and further to use biotechnological methods to remediate the soil polluted by OPs.

One perspective method to biologically clear polluted soil is phytoremediation. We studied plant community structure in areas surrounding each hot point to describe botanical diversity and to identify pesticide-tolerant plant species that may be useful for phytoremediation.

#### Identification of pesticide-tolerant plant species at hot points by surveys of plant community structure

The diversity of plant populations growing at hot points allowed us to identify pesticide-tolerant species. At the first hot point, 75 plant species from 26 families were documented. At the second point, 83 species from 23 families and at the third point, 87 species from 22 families were observed (Nurzhanova *et al.* 2004). Seventeen pesticide-tolerant species were identified: *Artemisia annua* L. Sp. Pl., *Artemisia absinthium* Willd., *Agropyron pectibiform* L., *Artemisia proceraeformis* L., *Amaranthus retroflexus* L., *Ambrosia artemisiifolia* L., *Barbarea vulgaris* R Br., *Bromus tectorum* Leyss, *Erigeron canadensis* L., *Kochia scoparia* schrad, *Kochia sieversiana* L., *Lactuca tatarica* (L.) C.A. Meg., *Onopordon acanthium* L., *Polygonum aviculare* L., *Rubus caesius* DC, *Rumex confertus* L. and *Xanthium strumarium* L.

Distinctive features of these species included relatively high vegetative cover near the center of the hot points. These species expressed high phenotypic plasticity (flexible expression of morphological characteristics) that suggests adaptation to pesticide-contaminated conditions. These characteristics included plant height, root system development, root to shoot ratio, branching, color of leaves, and display of "gigantism" and "miniaturization" effects (**Fig. 5-6**).

### Greenhouse fate and transport study using soil from hot points

Pesticide-tolerant species were used to study the pattern of accumulation of pesticides in a greenhouse pot study. Seed of 13 plant species were grown in three soil treatments using soil from hot points 1 and 2, and a control soil, all in triplicate. All of the species established sufficiently to be used for analysis. Soil was sampled at the beginning of the experiment. It was confirmed that the soil from the site of Karasajsk district was polluted by OPs (Table 1). The average concentration (in µg/kg) of pesticides in the soil of

Point 1 was 734, Point 2 = 6268 and Point 3 = 343.

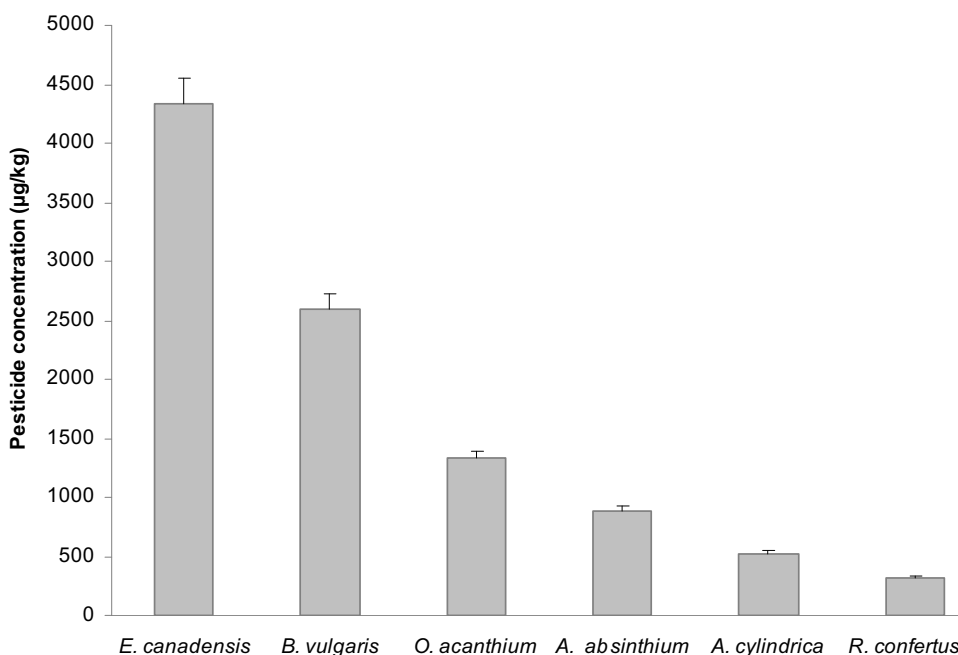
Plant tissue and soil were sampled at the time of flowering to estimate plant biomass production and content of HCH isomers and DDT metabolites in soil, plant root tissue, and above-ground plant tissue. In the greenhouse study, the amount of pesticides accumulated in plant tissue depended on the plant species, plant biomass production, and initial level of pesticide contamination in soil. Figs. 7 and 8 show the total concentration of pesticides in plant tissue for the 13 species grown in soil from hot point 2.

Four groups of plant species were identified based on the observed pattern of pesticide accumulation.

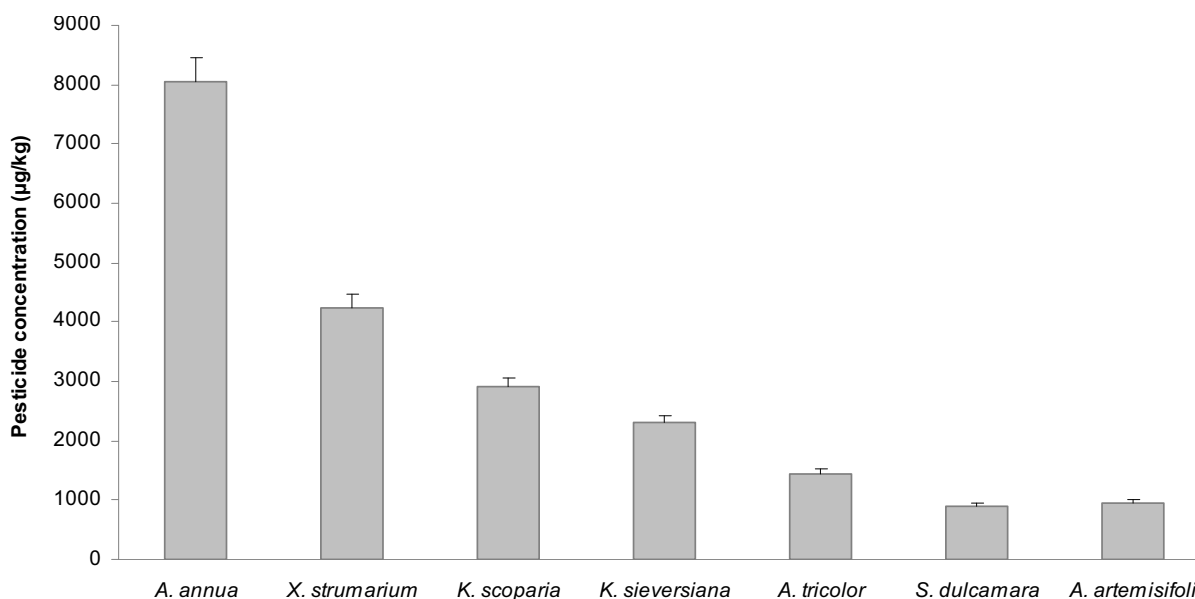
**Table 1** The middle concentrations of pesticides in soil from “hot points” (Karasajsk rayon). All concentrations are in µg/kg.

Hot points	α-HCH	β-HCH	γ-HCH	4,4-DDE	2,4-DDD	4,4-DDT	Sum of pesticides
MAC	0	100	100	100	0	100	
Point 1	67.1 ± 9.1	176.0 ± 23.3	22.2 ± 3.2	85.1 ± 3.4	19.3 ± 3.2	365.2 ± 36.3	734.9
Point 2	15.3 ± 7.3	83.2 ± 5.5	13.0 ± 4.2	1869.0 ± 102.1	101.1 ± 16.2	4187.0 ± 284.3	6268.6
Point 3	0	0	0	302.3 ± 20.5	0	41.3 ± 5.8	343.5

MAC Maximum Acceptable Concentrations



**Fig. 7** Total pesticide residuals accumulated in plant tissue for six biannual plant species grown in soil from Karasajsk district of Almaty oblast (hot point 2). Values represent mean ± Standard Error (SE).



**Fig. 8** Total pesticide residuals accumulated in plant tissue for seven annual plant species grown in soil from Karasajsk district of Almaty oblast (hot point 2). Values represent mean ± Standard Error (SE).

**Table 2** Relation amount of pesticides in an above-ground of plants to the amount of pesticides in root.

Species	TCF		
	Point 1	Point 2	Point 3
<b>Annual plants</b>			
<i>Artemisia annua</i>	1,1	0,89	0,53
<i>Ambrosia artemisifolia</i>	0,31	0,12	0,25
<i>Xanthium strumarium</i>	0,50	0,81	0,18
<i>Kochia scoparia</i>	0,26	0,11	0,33
<i>Kochia Sieversiana</i>	0,14	0,26	0,41
<i>Amaranthus tricola cult.</i>	0,04	0,15	0,12
<i>Solanum dulcamara</i>	0,04	0,02	-
<i>Medicago sativa</i>	0,03	0,15	0,01
<b>Bi-annual plants</b>			
<i>Rumex confertus</i>	0,29	0,03	0,65
<i>Erigeron canadensis</i>	0,31	0,68	0,65
<i>Aelgilops cylindrical</i>	0,03	0,06	0,15
<i>Artemisia absinthium</i>	0,02	0,11	0,11
<i>Barbarea vulgaris</i>	0,04	0,27	0,2

TCF Factor translocation pesticides from root to in above-ground of plants

1. Pesticide-accumulating plants: These are species in which the residual concentration of OPs in plant tissues exceeded the MAC by 400 times (maximum concentration limit for plants in Kazakhstan is 20 µg/kg). Species in this category include: *Xanthium strumarium*, *Kochia scoparia*, *Artemisia annua* and *Kochia sieversiana*.

2. Accumulators of HCH isomers. These are species in which the residual concentration of HCH isomers in plant tissues exceeds the MAC value by 90 times. Four representatives of the *Asteraceae* family are in this category: *Artemisia annua*, *Ambrosia artemisifolia*, *Xanthium strumarium*, and *Erigeron canadensis*.

3. Accumulators of 2,4 DDD and αHCH metabolites. These compounds do not have a maximum allowable concentration for plants or soil. These species accumulate trace metabolites of 2,4 DDT and αHCH in plant tissues in which the residual concentration of pesticides exceeds the MAC for other compounds. These species include *Ambrosia artemisifolia*, *Xanthium strumarium*, *Artemisia annua*, *Solanum dulcamara*, *Medicago sativa* and *Barbarea vulgaris*.

4. Non-accumulators. These are plant species that accumulate insignificant concentrations of pesticides in plant tissues. These species include *Solanum dulcamara* and *Rumex confertus*. They grow in the central zone of the “hot spots” in pesticide-contaminated soil. These species may have practical value for development of phytoremediation technologies and should be investigated further for their ability to stabilize or enhance degradation of OPs in soil.

Histological methods were used to locate OP residues in plant tissue. The results of this analysis demonstrated pesticides were distributed unevenly within different plant tissues. If a species had a dorsiventral and isolateral leaf type, then pesticides appeared to accumulate in the palisade mesophyll. If the species had homogeneous mesophyll, then pesticide residues collected in mesophyllous cells around conducting bunches (Table 2). For example, *Xanthium strumarium* L. has a dorsiventral type of leaf; thus, pesticides collected in the palisade mesophyll. In the stem, pesticides collected in walls of xylem cells. In root tissue, pesticides collected in parenchymous cells and xylem walls.

One of the requirements for phytoremediation is ability of a substance to migrate from roots to the above-ground

part of plants. To assess the intensity of migration of pesticides in the “soil-root-above-ground” system the translocation factor was used (Table 3). The coefficient of the translocation factor depended on the concentration of pesticides in soil. For example, the translocation factor for *Artemisia annua* varied from 0.53 to 1.1.

The basic organ of accumulation of pesticides among plant tissues is the root system. Most pesticides are accumulated in the root system, although among the species we investigated some species which demonstrated an ability to transfer pesticides from roots to above-ground tissues: *Artemisia annua*, *Xanthium strumarium*, *Erigeron canadensis* and *Rumex confertus*.

Total pesticide accumulation was highest in *Xanthium strumarium*, accumulating 2.9 to 78.4 µg of pesticide. Other ranges of pesticides accumulated were (in µg): *Artemisia annua* (1 to 42.4); *Kochia scoparia* (6.40 to 23), *Ambrosia artemisifolia* (from 2.9 to 13.8), *Kochia sieversiana* (1.9 to 25.0) and *Solanum dulcamara* (1.09 to 43). On the basis of this data it is possible to assume that the cumulative ability of tolerant plants is an adjustable process. By increasing the biomass of plants it is possible to increase the accumulation of pesticides in the vegetative body of plants.

## CONCLUSIONS

From the results obtained in our work, it can be concluded that sites of former storehouses in Kazakhstan (Almaty oblast) are new original centers of contamination, or “Hot Points”. Our research has shown the presence of OPs (metabolites of dichlorodiphenyltrichloroethane and isomers of hexachlorocyclohexane) as hazardous substances in the soil around 24 former pesticide storehouses where their concentration exceeded MAC value by tens - hundreds times. In “Hot points” has revealed 17 species tolerant to pesticides. It is investigated that wild species may be useful for phytoremediation of pesticide contaminated soil. Tolerant plants have the high ability to accumulate pesticides in tissues and migration ability of pesticides in system “soil – root – stem – leaves”.

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**Table 3** Localization of chlororganic pesticides in plants.

Species	Type of mesophyll	Roots	Stems	Leaves
<i>Xanthium strumarium</i> L.	Dorsiventral	In parenchymous cells and xylem walls	Xylem walls	Palisade mesophyll
<i>Ambrosia artemisifolia</i> L.	Isolateral	In parenchymous cells and xylem walls	Xylem walls	Palisade mesophyll
<i>Erigeron canadensis</i> L.	Dorsiventral	In parenchymous cells and xylem walls	Xylem walls	Palisade mesophyll
<i>Artemisia annua</i> L.	Homogeneous	In parenchymous cells and xylem walls	Xylem walls	Around conducting bunches
<i>Kochia scoparia</i> L.	Homogeneous	In parenchymous cells and xylem walls	Xylem walls	Around conducting bunches
<i>Barbarea vulgare</i> L.	Dorsiventral	In parenchymous cells and xylem walls	Xylem walls	Palisade mesophyll

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