Obsolete pesticides and application of colonizing plant species for remediation of contaminated soil in Kazakhstan

Asil Nurzhanova, Sergey Kalugin & Kabl Zhambakin

Environmental Science and Pollution Research

ISSN 0944-1344

Environ Sci Pollut Res
DOI 10.1007/s11356-012-1111-x

ONLINE FIRST

Springer

Environ Sci Pollut Res
DOI 10.1007/s11356-012-1111-x

Obsolete pesticides and application of colonizing plant species for remediation of contaminated soil in Kazakhstan

Asil Nurzhanova · Sergey Kalugin · Kabl Zhambakin

Received: 7 May 2012 / Accepted: 24 July 2012

Abstract In Kazakhstan, there is a problem of finding ways to clean local sites contaminated with pesticides. In particular, such sites are the deserted and destroyed storehouses where these pesticides were stored; existing storehouses do not fulfill sanitary standards. Phytoremediation is one potential method for reducing risk from these pesticides. Genetic heterogeneity of populations of wild and weedy species growing on pesticide-contaminated soil provides a source of plant species tolerant to these conditions. These plant species may be useful for phytoremediation applications. In 2008–2009 and 2011, we surveyed substances stored in 80 former pesticide storehouses in Kazakhstan (Almaty oblast) to demonstrate an inventory process needed to understand the obsolete pesticide problem throughout the country, and observed a total of 354.7 t of obsolete pesticides. At the sites, we have found organochlorine pesticides residues in soil including metabolites of dichlorodiphenyltrichloroethane and isomers of hexachlorocyclohexane. Twenty-four of the storehouse sites showed pesticides concentrations in soil higher than maximum allowable concentration which is equal to 100 $\mu g\ kg^{-1}$ in Kazakhstan. Seventeen pesticide-tolerant wild plant species were selected from colonizing plants that grew into/near the former storehouse's pesticides. The results have shown that colonizing plant annual and biannual species growing on soils polluted by pesticides possess ability to accumulate organochlorine pesticide residues and reduce pesticide concentrations in soil. Organochlorine pesticides taken up by the plants are distributed unevenly in different plant tissues. The main organ of organochlorine pesticide accumulation is the root system. The accumulation rate of organochlorine pesticides was found to be a specific characteristic of plant species and dependent on the degree of soil contamination. This information can be used for technology development of phytoremediation of pesticide-contaminated soils.

Keywords Obsolete pesticides · Phytoremediation · Wild plant · Dichlorodiphenyltrichloroethane · Hexachlorocyclohexane

Introduction

Kazakhstan celebrated its independence from the former Soviet Union in 1991; however, the impending environmental problems were not anticipated. Within 5 years of independence, pesticide storage warehouses from the official plant protection service of the former Soviet Union (called "Agrochemservice") were destroyed leaving the stored obsolete pesticides and their containers unattended and open to the environment. Most of the bulk pesticides have been moved to other storage areas, taken by citizens for individual use or resale in labeled or unlabeled containers, or released into the surrounding environment with no indication of their potential danger to local residents. However, people living around these warehouses use the land for pasture, kitchen gardens, play areas for children, and a source of construction materials. In Kazakhstan, pollution of soil and water by obsolete pesticides is a serious ecological problem.

The areas of these former storehouses have become "hot spots" of contamination and represent a serious ecological danger. The largest former storehouses of "Agrochemservice" were located in Almaty region because of the administrative importance of these areas and the level of agricultural development. Official data

Responsible editor: Philippe Garrigues

A. Nurzhanova (✉) · S. Kalugin · K. Zhambakin
Institute of Plant Biology and Biotechnology,
45 Timiryazev st.,
Almaty 050040, Kazakhstan
e-mail: gen_asil@mail.ru

Published online: 14 August 2012

on the number of stocks location and quantities of buried or unburied pesticides are inconsistent. For example, the Ministry for Environmental Protection estimates that in Almaty region more than 87 t of pesticides must be buried, while the Ministry of Agriculture estimates about 126 t of pesticides to be buried (Nazhmetdinova 2001).

Kazakhstan signed and ratified the Stockholm, Rotterdam, and Basel Conventions showing that the Republic has taken an important step towards integration into the global process of cooperation in the field of human health and the environment. In 2004, a Global Environment Facility-sponsored project to provide initial support for the performance of Kazakhstan's obligations under the Stockholm Convention estimated there were 10,000 t of obsolete pesticides and pesticide mixtures. Among the identified pesticides, persistent organic pollutants (POPs) were not detected. It is expected that POPs pesticides may be present among the unidentified mixtures (UNEP 2008). The inventory of obsolete pesticides remains one of the most difficult problems for Kazakhstan.

Published data on actual concentrations of organochlorine pesticides in soils of Kazakhstan is very poor. Linker (2000) reports that average concentration of dichlorodiphenyltrichloroethane (DDT) and its metabolites in Almaty region is 0.32 kg km^{-2}. The data on concentrations of obsolete pesticides in former warehouses is unavailable. Preliminary analyses of soils around these sites showed the presence of pesticides of DDT and hexachlorocyclohexane (HCH) groups in concentrations up to 62 mg kg^{-1} (Nurzhanova et al. 2004a). There has been insufficient scientific study to estimate the danger to public health and the environment from these sites.

One of the most efficient methods to cleanup soils contaminated with obsolete pesticides is the incineration in special high-temperature stoke (http://agrochemicals.iupac.org; fao.org/fileadmin/user_upload/o). However, such methods require high capital investments and generally too costly for developing countries like Kazakhstan. 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. Phytotechnologies use vegetation to accumulate, degrade, or stabilize environmental contaminants in soil, sediments, surface water, or groundwater (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). Two major mechanisms of phytoremediation for organic pesticides are rhizodegradation and phytoextraction (Pascal-Lorber and Lauren 2011).

The objective of this study was to identify pesticide-tolerant plant genotypes which can be used for phytoremediation of pesticide-contaminated soil in Kazakhstan. In this study, we focused on the following organochlorine pesticides: DDT and HCH along with their associated metabolites and isomers: 2,4′-DDD (2,4′-dichlorodiphenyldichloroethane); 4,4′-DDD; 4,4′-DDT; 4,4′-DDE (2,4′-dichlorodiphenyldichloroethylene); α-HCH; β-HCH; and γ-HCH. 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 study included the following tasks:

- Documenting obsolete pesticide stockpiles in former storehouses and characterization of soil contamination levels
- Identification of pesticide-tolerant plant species at "hot spots" by surveys of plant community structure
- Study of accumulation of pesticides by the selected pesticides tolerant plants in greenhouse conditions
- Histological analysis to locate pesticides in plant tissue
- Study the fate and transport of pesticides in soil and plants in the greenhouse
- Study of the effect of fertilization on phytoremediation potential in the field

Materials and methods

Documenting obsolete pesticide stockpiles in former storehouses and characterization of soil contamination levels

We surveyed obsolete pesticide storehouses in 10 of 14 districts in Almaty region. In each region, 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. We refer to the former storehouse sites where we have observed pesticide contamination as "hot spots."

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 organochlorine pesticides as a marker of field contamination. We took 800 soil samples to determine residual concentrations of organochlorine pesticides. Three replications were taken at each sampling. Residual concentrations of organochlorine pesticides in soil were determined using standard methods adopted by the United States Environmental Protection Agency using a gas chromatograph 6890 (Hewlett Packard, USA) equipped with an electron capture detector and a capillary column using States Environmental Protection Agency method 8081 B (USEPA 2007). Samples of soils were extracted in a Soxhlet apparatus (Supelco, USA) for 16 h with 300 mL methylene chloride. The extract was concentrated using Kuderna–Danish evaporative concentrator (Supelco, USA) to 1 mL and the solvent was exchanged to hexane with final volume of 1 mL. A sample was transferred to a vial for analysis by gas chromatography/electron capture detector to determine the composition and quantity of total DDT (SDDT) and total HCH (SHCH) in soils. Concentration of SDDT and SHCH in soils was calculated from SDDT standards run with each batch of samples by gas chromatography. Results obtained for 2,4′-DDD, 4,4′-DDT, 4,4′-DDE, α-HCH, β-HCH, and γ-HCH were expressed as microgram per kilogram of pesticides per gram dry weight of soil.

Identification of pesticide-tolerant plant species at "hot spots" by surveys of plant community structure

To identify pesticide-tolerant plant species, plant community structure was investigated at three former storehouse sites (Karasai district of Almaty region). The first point (point 1) is located 15 km from Almaty with an area of 80 m^2. 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^2. 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 time 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^2. Point 3 differs from points 1 and 2 in that there were remnants of old pesticide containers found at this site. At each location, plant species were identified along 400 mr transects originating from the center of each site. Plant community structure was described by the Tahtadjan (1987) technique. In the field, the following parameters of plant community structure were recorded: plant species identity, botanical family, patchiness, vegetative coverage, frequency, and distribution (Bykow 1978).

The study of soil and morphological description of separate horizons on 20-m distance from sites was made. The depth of soil for different points was various. For physicochemical characteristics of the studied soils, we used Kjeldal's, Geisler–Maksimyuk, and potentiometric methods (Nikolskiy 1965).

Study of accumulation of pesticides by the selected pesticides tolerant plants in greenhouse and field

Pesticide-tolerant species were used to study the pattern of accumulation of pesticides in a greenhouse pot study. Thirteen pesticide-tolerant plant species were grown in greenhouse containers along with a control treatment without vegetation. Each experimental unit was a container with 3 kg of clean or contaminated soil that had been placed above a layer of ceramzite clay and sand to facilitate drainage. Four soil treatments were used including a clean soil control; three soils collected from points 1, 2, and 3 in Karasai district of Almaty region. Each treatment was grown in three replications (13 species × 5 soils × 3 replications). Soil was sampled at the beginning of the study and after harvest of plants at flowering or approximately 6 months. Plant tissues (10 plants from each site) were to estimate plant biomass production and content of SHCH and SDDT in root and aboveground plant tissue.

In field, at each spot (located at the storehouses in Karasai and Talgar districts), two 1 × 1 m test sites were setup to study the effect of added fertilizer on phytoextraction by *Xanthium strumarium*. Species of *X. strumarium* was chosen because it was one of the dominant species occurring at the former storehouse sites with high biomass production (up to 0.0185 kg), a short vegetative period (up to 44 day), and demonstrated ability to accumulated metabolites of DDT and isomers of HCH (up to 4,250 μg kg^{-1}). It is also poisonous and not consumed by livestock (Kuosev 2000).

In Karasai district, 402 kg of soil was used to form each site, in Talgar district—200 kg. Initial total soil pesticide concentration in the field site of Karasai district was 489 μg kg^{-1}; total mass of pesticides in the field plots was 196,600 μg. Initial soil pesticide concentration in the field site of Talgar district was 7,291 μg kg^{-1}, total mass of pesticides in the field site was 1 458,200 μg. At one site, *X. strumarium* was grown with added fertilizer (20 g ammonium phosphate and 20 g potassium chloride per site), and the other site had *X. strumarium* growing without addition of fertilizer.

Residual concentrations of organochlorine pesticides in plant tissue were determined by the method 8250A (US EPA 2007) using 6890 gas chromatograph (Hewlett Packard, USA) equipped with an electron capture detector and a DB-5 capillary column (J&W Scientific, USA). Samples of plants were extracted by 250 mL of methylene chloride in a Soxhlet apparatus for 4–6 h. The extract was concentrated in Kuderna–Danish evaporative concentrator for 15–20 min to a final volume of 1 mL. Then, the solvent was exchanged to hexane. The extract was applied to a Florisil column and was flushed through with 10 mL of hexane and then concentrated down to 0.5 mL by nitrogen blow up. A 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 4,4′-DDT, 4,4′-DDE, 2,4′-DDD, α-HCH, β-HCH, and γ-HCH were expressed as microgram per kilogram of pesticides per gram wet weight of plants.

Methods of histology were used to confirm organochlorine pesticides localizations in plant tissues. In period of plant flowering, stems, leaves, and roots were cut to 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 freezing microtome TOS-2. Sampling was done to 15 sections of each organ of the plant species per site. Examinations of stems, leaves, and roots transverse sections were made with MBI-6 microscope fixed with a Zorki camera (Prodina 1960; Esau 1980).

Statistical analysis of data

As estimated criteria of accumulative ability of plants following parameters were used: the residual amount of pesticides in above-ground and root of plants, in microgram per kilogram; phytoexctraction was calculated as accumulation of pesticides in above-ground or root (the residual amount of pesticides in tissue of plants multiplied on wet weight), in microgram; and translocation factor was calculated as [SDDT]+[SHCH] (above ground)/[SDDT]+[SHCH] (in roots) to reflect the amount of contaminant that is transported to the shoots with respect to the amount that is in the roots. The statistical processing of our data was carried out using MS EXCEL software.

Results

Documenting obsolete pesticide stockpiles in former storehouses and characterization of soil contamination levels

We have found a total of 80 former storehouses (2009–2011). Several different classes of substances were identified. Much of the bulk chemical substances did not have readable labels and remain unidentified. The following classes of pesticides were observed: triazine herbicides (atrazine, protrazine, propazine, simazine), organophosphate insecticides (metaphos or methyl parathione), organochlorines (nitrophen and illoxan or diclofop-methyl), dinitroanaline herbicides (treflan), carbamate (temik or aldicarb), and a pesticide mixture including compounds labeled Thiram and Hataonyag. Total amount of identified obsolete pesticides was 36,620 kg. The amount of identified pesticides that are forbidden or banned was 350 kg. The quantity of unidentified mixtures of obsolete pesticides was 317,700 kg or 89.5 % of the total obsolete pesticide stockpiles (Table 1).

As we noted, territories of the former warehouses are in private hands. Some owners cleaned their territory from obsolete pesticides and disposed it to an unknown location. For example, in 2009, we discovered 30 t of obsolete pesticides in Talgar district; and in 2011, the amount of obsolete pesticides reduced to 17 t. So, screening pesticide-polluted sites will provide a base for development of an action plan to prevent or minimize ecological risk from pesticide pollution in Kazakhstan.

Table 1 The amount of obsolete pesticides in former warehouses in Almaty region

Districts	The amount of storehouses	The amount of obsolete pesticides (kg)	The amount of identified obsolete pesticides (kg)	Forbidden pesticides (kg)
Karasai	6	1,150	–	–
Enbekshi-Kazakh	18	7,870	30,600	–
Talgar	14	30,600	200	–
Dzhambul	5	100,400	2,950	350
Uigur	7	3,830	970	–
Balkash	7	500	–	–
Ili	3	107,150	1,450	–
Eskeldy	12	103,225	50	–
Kerbulak	8	–	–	–
Koksu	No	–	–	–
Total	80	354,725	36,620	350

Research showed the presence of organochlorine pesticide as hazardous substances in soil around 24 former storehouses of pesticides where their concentration exceeds maximum allowable concentration (MAC) 10–100× fold (MACs in soil of Kazakhstan is established at the level of 100 μg kg^{-1}). In soil around 21 former warehouses, pesticides were not discovered; but in soil around 19 warehouses of the pesticides, concentration of pesticides was below MAC. The basic pollutants were isomers of α-HCH, β-HCH, and metabolites of 4,4′-DDE, 4,4′-DDD (Table 2).

Table 2 Quantities of the former storehouses polluted by organochlorine pesticides in Almaty region

Districts	The amount of storehouses	The amount of unpolluted storehouses	The amount of storehouses polluted with pesticides (lower than MAC)	The amount of storehouses polluted with pesticides (higher than MAC)
Karasai	6	0	0	6
Talgar	7	1	1	5
Dzhambul	5	1	2	2
Enbekshi-Kazakh	9	0	2	7
Uli	3	1	1	1
Uigur	7	4	3	0
Balkazh	7	2	4	1
Kerbulak	8	8	0	0
Eskeldy	12	4	6	2
Koksu	0	0	0	0
Total	64	21	19	24

These data show the ecological danger of these areas and also represent a significant potential risk to nearby populated areas. It is necessary to take urgent measures on liquidation of first of all obsolete pesticides and further use biotechnological methods to remediate the polluted soil by organochlorine pesticide. One perspective method to biologically cleanup to reduce the risk from these pesticides is phytoremediation. So, we studied plant community structure in areas surrounding each point to describe botanical diversity, identify pesticide-tolerant plant species that may be used for phytoremediation, and to understand the mechanisms of detoxification of soil by plants.

Identification of pesticide-tolerant plant species at "hot spots" by surveys of plant community structure

Selection of plant species is a critical decision for successful application of phytotechnologies. We documented plant community structures at the hot spots and identified plant species that grow in pesticide-contaminated soil near the center of the sites (three former storehouses located in Karasai district of Almaty region). The type of vegetation was characterized as early succession plant species dominated by annuals and biannuals. Many of the species would typically be considered as weeds. At point 1, 75 plant species from 26 families were documented; at the second spot, 83 species from 23 families and at the third spot, 87 species from 22 families were observed (Nurzhanova et al. 2004b).

Seventeen pesticide-tolerant species were identified in three of the former storehouses located in Karasai district of Almaty region including: *Artemisia annua* L. Sp. Pl., *Artemisia absinthium* Willd., *Agropyrom pectiboform* 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., Meg., *Onopordon acanthium* L., *Polygonum aviculare* L., *Rubus caesius* DC, *Rumex confertus* L., and *X. strumarium* L.

As the result of study of vegetation on territory of the former storehouses (40 sites) of other districts (Enbekshi-Kazakh, Talgar, Dzhambul, and Ili with similar climatic condition), we have confirmed that there are pesticide-tolerant plant species. Soils in these districts are foothill chestnut calcareous. Distinctive features of these species included relatively high vegetative cover near the center of the hot spots. 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.

The amount of residual pesticides in tissues of tolerant plants growing in soil polluted by pesticides in a greenhouse

It was confirmed that the soil from the site of Karasai district was polluted by organochlorine pesticides. Average total concentration of organochlorine pesticides in the soil pattern of point 1 was 734 μg kg^{-1}, point 2 - 6,268 μg kg^{-1}, and point 3 - 343 μg kg^{-1} (Table 3). The most polluted point is point 2 where the concentrations of organochlorine pesticides exceeded MAC 10× fold. Maximum pesticide concentrations observed in soil samples were at concentrations of 4,187± 284 μg kg^{-1} for 4,4′-DDT and 1,869±102 μg kg^{-1} for 4,4′-DDE. At point 1, the primary contaminants were 4,4′-DDT and β-HCH. Soil concentrations exceeded MACs by 1.7–3.6 times. Results for point 3 were similar to point 1.

The presence of α-HCH and 2,4′-DDD at points is of special concern according to normative standards for Kazakhstan, their presence in soil is not permitted The contents of α-HCH at spots 1 and 2 varied from 15.3±7.3 to 67±9 μg kg^{-1}. Concentrations of 2,4′-DDD ranged from 19.3±3.2 to 101.1±16 μg kg^{-1}. These data show the ecological danger of these areas also represent a significant potential risk to nearby populated areas.

Soils in "hot spots" was foothill chestnut calcareous alkaline. The soil of point 1 (depth 0–80 cm) had content of 1.3–2.6 % $CaCO_3$, 0.15–0.18 % nitrogen, 0.12–0.24 % phosphorus, 2.7–3.0 % potassium, 1–1.8 % humus, and 7.9–8.2 pH. The soil of point 2 (depth 0–70 cm) had content of 8.2–13.7 % $CaCO_3$, 0.1–0.2 % nitrogen, 0.1–0.2 % phosphorus, 2.5–2.7 %

Table 3 The middle concentrations of pesticides in soil from "hot spots" (Karasai district)

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	n/a
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	1,869.0±102	101.1±16	4,187.0±284	6,268.6
Point 3	n/d	n/d	n/d	302.3±20.5	n/d	41.3±5.8	343.5

All concentrations are in microgram per kilogram. Soil was foothill chestnut calcareous alkaline

n/a not available, *n/d* not detected

potassium, 8.1–13.7 % humus, and 8.2–8.3 pH. Soil of point 3 (depth 0–40 cm) had content of 6.3–12.9 % $CaCO_3$, 0.08–0.2 % nitrogen, 0.08–0.18 phosphorus, 2.4–2.5 % potassium, 8.1–8.3 % humus, and 8.1–8.3 pH.

It has been established that that the amount of pesticides accumulated in plant tissue depends on the plant species, plant biomass production, and initial level of pesticide concentration in soil. Figures 1 and 2 show the total concentration of pesticides in plant tissues for the 13 species grown in soil from point 2. Five groups of plant species were identified based on the observed pattern of pesticide accumulation.

The concentration of pesticides in plant tissues was found to be 400 times higher than MAC value (MAC for plant tissue in Kazakhstan is 20 μg kg^{-1}). Species in this category include *X. strumarium*, *K. scoparia*, *A. annua*, and *K. sieversiana*. In the period of flowering, the length of *X. strumarium* was 42 cm and wet biomass was 0.020 kg; the length of *K. scoparia* was 33 cm and wet biomass was 0.0079 kg; the length of *A. annua* was 34 cm and wet biomass was 0.0053 kg; the length of *K. sieversiana* was 37 cm and wet biomass was 0.011 kg.

The concentration of HCH isomers in plant tissue was found to be 90 times higher than MAC. Four representatives of family *Asteracea* in this category include *A. annu*a, *A. artemisiifolia*, *X. strumarium*, and *Erigeron canadensi*s. In the period of flowering length, *A. artemisiifolia* was up to 38 cm, and wet biomass was 0.020 kg; the length of *E. canadensi*s was 30 cm and wet biomass was 0.004 kg.

These species accumulate trace metabolites of DDT and α-HCH in plant tissues in which residual concentration of pesticides exceeds MAC for other compounds. These species include *A. artemisiifolia*, *X. strumarium*, *A. annu*a, *Solanum dulcamara*, *Medicago sativa*, and *B. vulgaris*. In the period of flowering, the length of *S. dulcamara* was up to 86 cm and wet

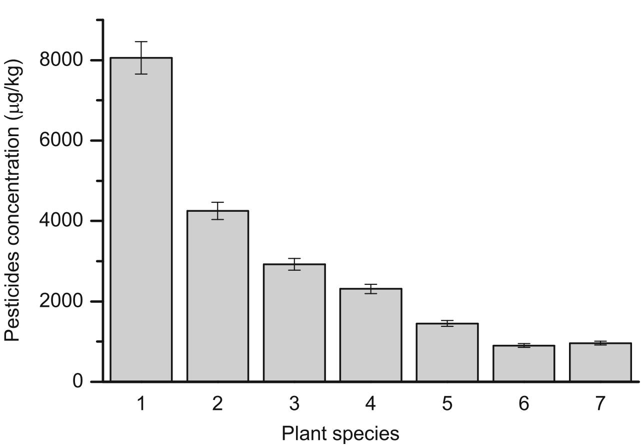

Fig. 1 Total pesticide residuals accumulated in plant tissue for seventh annual plant species grown in soil from Karasai district Almaty region (point 2). Values represent mean±SE. Plant species: *1 A. annua*, *2 X. strumarium*, *3 K. scoparia*, *4 K. sieversiana*, *5 Amaranthus tricola*, *6 S. dulcamara*, *7 A. artemisiifolia*

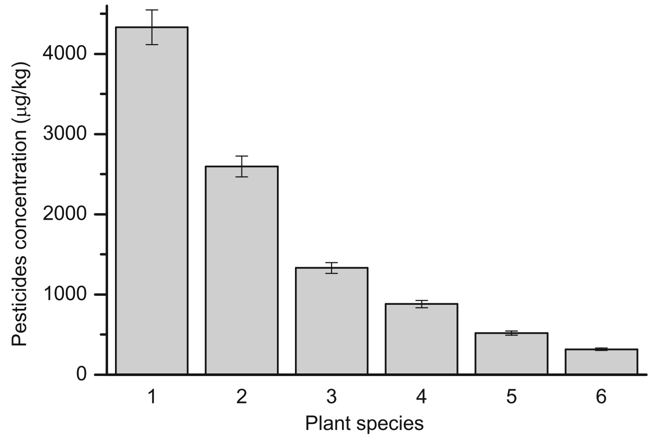

Fig. 2 Total pesticide residuals accumulated in plant tissue for six biannual plant species grown in soil from Karasai district of Almaty region (point 2). Values represent mean±SE. Plant species: *1 E. canadensis*, *2 B. vulgaris*, *3 Opordon acanthium*, *4 A. absinthium*, *5 Aegilops cylindrica*, *6 R. confertus*

biomass was 0.048 kg; the length of *M. sativa* was 25 cm and wet biomass was 0.0011 kg; and the length of *B. vulgari*s was 35 cm and wet biomass was 0.004 kg.

Most pesticides accumulate in the root system; however, some species demonstrated capability to translocate pesticides from roots to aboveground tissues. These included *K. scopari*a, *A. annu*a, *B. vulgaris*, and *A. artemisiifolia.* For these plants, concentration of pesticides in aboveground tissue exceeded concentration in root tissue, giving a translocation factor of greater than 1.

Two species, *S. dulcamara* and *R. confertus*, did not accumulate significant concentrations of pesticides in plant tissues despite growing in the most contaminated areas of the hot points. These species may have practical value for phytostabilization or phytodegradation technologies that seek to stabilize or enhance degradation of organochlorine pesticides in soil. In the period of flowering, the length of *R. confertus* was up to 32 cm and wet biomass was 0.021 kg.

One of the requirements to phytoremediation use is ability to migrate from root to above-ground of plants. For definition of intensity of migration of pesticides in system "soil–root–above-ground", the translocation factor was used (Table 4). Most pesticides are accumulated in the root system; however, among the studied species, some species demonstrated ability to transfer pesticides from roots to the above-ground tissues. They are *A. annua*, *X. strumarium*, *E. canadensis*, and *R. confertus.* Translocation factor of pesticides from root to aboveground plant depended on concentration of pesticides in soil. For example, the species *A. annua* growing in soil from point 1 accumulated on aboveground 1,430±109 μg kg^{-1} and root 1,230±87 μg kg^{-1} (translocation factor 1.1); from point 2, on aboveground 3,775±154 μg kg^{-1} and root 4,283±123 μg kg^{-1} (translocation factor 0.89); and from point 3, on aboveground 268±

Table 4 Relation amount of pesticides in an above-ground of plants to the amount of pesticides in root

Species	TLF		
	Point 1	Point 2	Point 3
Annual plants			
A. annua	1.1	0.89	0.53
A. artemisiifolia	0.31	0.12	0.25
X. strumarium	0.50	0.81	0.18
K. scoparia	0.26	0.11	0.33
Kochia Sieversiana	0.14	0.26	0.41
Amaranthus tricola cult	0.04	0.15	0.12
S. dulcamara	0.04	0.02	–
M. sativa	0.03	0.15	0.01
Bi-annual plants			
R. confertus	0.29	0.03	0.65
E. canadensis	0.31	0.68	0.65
Aelgilops cylindrical	0.03	0.06	0.15
A. absinthium	0.02	0.11	0.11
B. vulgaris	0.04	0.27	0.2

TLF translocation factor of pesticides from root to in above-ground of plants

22 μg kg^{-1} and root 468±87 μg kg^{-1} (translocation factor 0.53),

Total pesticide accumulation for selected plant species highly varied, *X. strumarium* demonstrated pesticide accumulation from 2.9 to 78.6 μg. Other range of pesticide accumulation by plant species included: *A. annua* (1–42.7 μg); *K. scoparia* (6.40–23 μg), *A. artemisiifolia* (2.9–13.8 μg), *K. sieversiana* (1.9–25.4 μg), and *S. dulcamara* (1.09–43 μg).

On the basis of these data, it is possible to assume that accumulative ability of tolerant plants is adjustable process. By increasing plant biomass, it is possible to increase the accumulation of pesticides in the vegetative body of plants.

Histological analysis to locate pesticides in plant tissue

Histological methods were used to locate organochlorine pesticide residues in plant tissue (Prodina 1960; Esau 1980; Karthikeyan et al. 2004). The results of this analysis demonstrated that 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. For example, *X. strumarium* L. has a dorsiventral type of leaf; thus, pesticides are collected in the palisade mesophyll. In the stem, pesticides collected in walls of xylem cells. In root tissue, pesticides are collected in parenchymous cells and xylem walls (Table 5).

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

Any technology to reduce risk from pesticide-contaminated soil must track the fate of toxic compounds using a mass balance approach. The purpose of this study was to study the fate of pesticides in soil with and without plants. Thirteen pesticide-tolerant plant species were grown in greenhouse containers along with a control treatment without vegetation.

For treatments with no plants, overall soil pesticide concentrations decreased 41–44 % for contaminated soil treatments from points 1, 2, and 3. This decrease in pesticide concentrations was due to a combination of possible natural breakdown of pesticide compounds and migration of compounds. This result illustrates the difficulty in tracking pesticide fate in these studies. Some of the compounds migrated into the sand layer of the containers. The sand was clean at the beginning of the study.

For the 13 plant species tested, reduction of soil pesticide concentrations ranged from about 30 to 80 % (Fig. 3). The amount of pesticides accumulated in plant tissues was a small proportion of the total dissipation. Of the 13 species tested, *S. dulcamara* accumulated the largest amount of pesticide, 177 g or 1.21 % of the original soil pesticide content from hot point 2. The percentage of pesticide reduced from phytoextraction in this experiment ranged 0.01–0.04 % for plants growing in

Table 5 Localization of pesticides in vegetative body of plants

Species	Type of mesophyl	Vegetative bogy		
		Root	Stem	Leave
X. strumarium L	Dorsiventral	In parenchymous cells and xylem walls	Xylem walls	Palisade mesophyll
Ambrosia artemisiafolia L.	Isolateral	In parenchymous cells and xylem walls	Xylem walls	Palisade mesophyll
E. canadensis L.	Dorsiventral	In parenchymous cells and xylem walls	Xylem walls	Palisade mesophyll
A. annua L.	Homogeneous	In parenchymous cells and xylem walls	Xylem walls	Around conducting bunches
K. scoparia L.	Homogeneous	In parenchymous cells and xylem walls	Xylem walls	Around conducting bunches
B. vulgaris L.	Dorsiventral	In parenchymous cells and xylem walls	Xylem walls	Palisade mesophyll

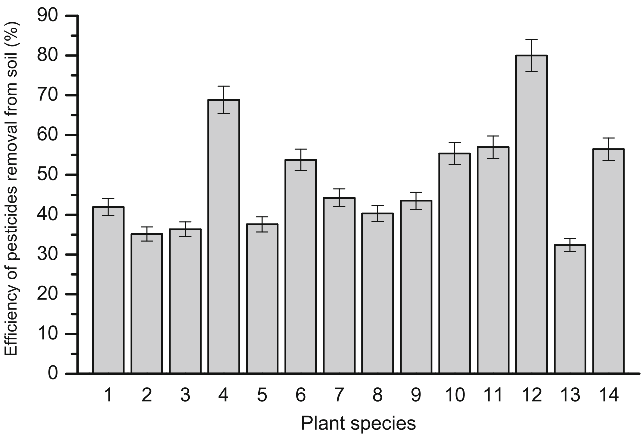

Fig. 3 Reduction of soil pesticide concentration with 13 annual and biannual plant species grown in soil from Karasai district Almaty region (point 2). Values represent mean±SE. Plant species: *1* control (the polluted soil without plants), *2 A. annua*, *3 A. artemisiifolia*, *4 X. strumarium*, *5 E. canadensis*, *6 A. absinthium*, *7 K. scoparia*, *8 K. sieversiana*, *9 B. vulgaris*, *10 Amaranthus tricolor*, *11 M. sativa*, *12 S. dulcamara*, *13 R. confertus*, *14 A. cylindrica*

point 1 soil; 0.01–1.2 % for plants growing in point 2 soil; and 0.01–0.1 % for plants growing in point 3 soil.

The experiment resulted in the following useful observations: the amount of pesticides taken up in plant tissue varies with initial soil pesticide concentrations and plant biomass produced; plant species appear to vary in the amount of pesticides residues they accumulate; some plant species are more useful for stabilization of pesticides in soil than for accumulation of pesticides in plant tissue; although soil pesticide concentrations in this study decreased by 30–80 % with different plant species treatments, only a small proportion of this decrease was due to phytoextraction; and good control of pesticide mass balances is needed to advance development of phytoextraction technologies.

Study of the effect of fertilization on phytoremediation potential in the field

In the field, experiments were conducted on the territory of the two former warehouses (Karasai and Talgar districts). Climatic conditions of these two districts are similar. Soil in Talgar district as well as Karasai district is foothill chestnut calcareous. We studied the effect of added fertilizer on phytoextraction by *X. strumarium* (Table 6).

Table 6 Pesticide concentrations and mass in soil and *X. strumarium* plants from two test plots at hot spots; one test plot had no added fertilizer and one test plot was fertilized with ammonium phosphate and potassium chloride

Sample	Soil or plant mass (kg)	Pesticide concentration (μg kg^{-1})						Pesticide mass (μg)
		α-HCH	β-HCH	γ-HCH	4,4′-DDE	2,4′-DDD	4,4′-DDT	
Karasai district								
Contaminated soil without fertilizer								
Soil before experiment	402	15±7	83±5	13±4	106±12	85±16	187±28	196.6
Aboveground	1.3	n/d	n/d	n/d	15±4	n/d	45±7	78
Root	0.1	8±2	49±5	7±2	24±6	n/d	94±8	18
Soil after experiment	402	2±1	39±4	4±1	89±8	17±4	76±9	91.3
Contaminated soil with fertilizer								
Soil before experiment	402	11±3	35±8	7±2	162±12	5±2	200±15	168.8
Aboveground	3.3	n/d	17±2	n/d	7±4	2	1	334
Root	0.3	14±2	10±2	10±1	156±6	1±0.1	283±15	142
Soil after experiment	402	4±2	6±1	10±1	9±1	1±0.1	83±5	45.4
Talgar district								
Contaminated soil without fertilizer								
Soil before experiment	200	n/d	309±47	n/d	3,080±240	n/d	3,902±297	1,458.2
Aboveground	0.140	n/d	n/d	n/d	359±120	n/d	1,862±422	170
Root	0.016	n/d	165±11	n/d	1,090±14	n/d	1,155±124	38
Soil after experiment	200	n/d	213±14	n/d	2,713±198	n/d	2,202±204	983.0
Contaminated soil with fertilizer								
Soil before experiment	200	n/d	309±47	n/d	3,080±240	n/d	3,902±297	1,458.2
Aboveground	0.224	n/d	13±6	n/d	229±33	n/d	2,746±629	239
Root	0.015	n/d	100±8	n/d	322±47	n/d	590±74	44
Soil after experiment	200	n/d	124±12	n/d	2,000±128	n/d	1,433±145	710.2

n/d not detected

Plants accumulated significant concentrations of pesticides into plant tissue compared to the initial concentrations in soil; however, the mass of pesticides taken up into plant tissue represents a very small fraction of the total pesticide mass in the soil. However, addition of fertilizer appeared to increase plant biomass production and increase the amount of pesticides accumulated in plant tissue. For example, *X. strumarium* growing on the polluted site (Karasai district) accumulated 334 μg (without fertilizer 78 μg) above-ground and -142 μg root (without fertilizer 18 μg). Plant biomass production increased from 1.3 to 3.3 kg.

We showed that species of *X. strumarium* (without fertilizer) accumulated 182 μg kg^{-1} in root and 60 μg kg^{-1} above-ground pesticides (stem, 9.7±0.9 μg kg^{-1}; leaves, 17± 2 μg kg^{-1}; blossom cluster, 13±0.8 μg kg^{-1}; and fruits, 20± 0.9 μg kg^{-1}). The *X. strumarium* species (with fertilizer) accumulated 473 μg kg^{-1} in root and 367 μg kg^{-1} above-ground pesticides (stem, 66±2 μg kg^{-1}; leaves, 367± 12 μg kg^{-1}; and fruits, 42±2 μg kg^{-1}).

The contents of pesticides in blossom cluster and fruits testify that organochloride pesticides transported through system: "root–stalk–leaves–blossom cluster–fruit". From the pesticide concentrations observed in soil, it was suggested that practically useful soil remediation processes may be functioning; however, mechanisms other than phytoextraction are apparently responsible for this change. Prior to bioremediation and phytoremediation studies with DDT and HCH, it was reported that transformations take place in soils under favorable conditions (Karthikeyan et al. 2004; Kamanavalli and Ninnekar 2004; Phillips et al. 2005; Huang et al. 2007; Raina et al. 2008).

Conclusion

From the results obtained in our work, it can be concluded that sites of former storehouses in Kazakhstan (Almaty region) are new original centers of contamination. Our research has shown the presence of POPs (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 10 times. These data demonstrate the potential ecological danger and health risk posed by the former pesticide storehouses, especially those located near populated areas. Resolution of this risk will require elimination of obsolete pesticide stockpiles and pesticide containers, including locations where pesticides have been buried. Further priorities include remediation of soil polluted by organochlorine pesticides. One of the promising innovative technologies for managing pesticide-contaminated soils is phytoremediation. For the development of technology for phytoremediation of soils around former pesticides storehouses, a flora was studied. In "Hot spots", there were 17 species tolerant to pesticides. A greenhouse experiment using the pesticide-tolerant species showed that certain plant species from the wild populations have the ability to accumulate organochlorine pesticides and their metabolites (4,4′-DDE, 2,4′-DDD, 4,4′-DDT, α-HCH, β-HCH, and γ-HCH). The accumulation rate of organochlorine pesticides was found to be a specific characteristic of plant species and dependent on the degree of soil contamination. Among the investigated species, concentrations of four accumulated metabolites of DDT and isomers of HCH in plant tissue exceeded the Kazakhstan's MAC for plant tissue by 90–400 times. Species in this category included: *A. annua*, *A. artemisiifolia*, *E. canadensis*, *K. sieversiana*, *K. scoparia*, and *X. strumarium*. 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 organochlorine pesticides in soil. It was stated that at growing of tolerant species on a polluted soil, pesticides concentration decreases by 30–80 %. It was noted that phytoextraction process for the studies species was minor although species of *S. dulcamara* accumulated the largest amount of pesticide, 177 g or 1.21 % of the original soil pesticide content from point 2. Field experiments using *X. strumarium* demonstrated that at optimization of the contaminated medium using the complex of fertilizers, biomass, phytoextraction potential of plant organism, and pesticides translocation from roots to above-ground part is decreased. This information can be used for technology development of phytoremediation of pesticide-contaminated soils. Further research is needed to understand the fate and transport of pesticides in these contaminated soils.

Acknowledgments This work was funded by the United States Environmental Protection Agency (US EPA) in the framework of the International Science and Technology Center (ISTC, Moscow) project K-750 and the Ministry of Education and Science of the Republic of Kazakhstan.

References

Bykow BA (1978) Geobotanics. Nauka, Almaty, In Russian

Cunningham SD, Anderson TA, Schwab AP, Hsu FC (1996) Phytoremediation of soils contaminated with organic pollutants. Adv Agron 56:55–114

Davis LC, Castro-Diaz S, Zhang Q, Erickson LE (2002) Benefits of vegetation for soils with organic contaminants. Crit Rev Plant Sci 21:457–491

Doty SL (2008) Tansley review: enhancing phytoremediation through the use of transgenics and endophytes. New Phytol 179:318–333

Dowling DN, Doty SL (2009) Improving phytoremediation through biotechnology. Curr Opin Biotechnol 20:204–206

Esau K (1980) Anatomy of seed plants. Wiley, New York

Huang Y, Zhao X, Luan S (2007) Uptake and biodegradation of DDT by 4 ectomycorrhizal fungi. Sci Total Environ 385:235–241

Kamanavalli CM, Ninnekar HZ (2004) Biodegradation of DDT by a *Pseudomonas* species. Curr Microbiol 48:10–13

Karthikeyan R, Davis LC, Erickson LE, Al-Khatib K, Kulakow PA, Barnes PL, Hutchinson SL, Nurzhanova AA (2004) Potential of plant-based remediation of pesticide-contaminated soil and water using non-target plants such as trees, shrubs, and grasses. Crit Rev Plant Sci 23:1–11

Kuosev P (2000) The full directory of plants. Moscow

Linker TV (2000) Preservation and burial of obsolete pesticides. Dissertation of PhD. Almaty. 26

Nazhmetdinova AS (2001) Pesticide and their application in Kazakhstan. Health Illness 1:36–39 (In Russian)

Nikolskiy NN (1965) Practical work on soil. Prakticum. Moskow: Education

Nurzhanova A, Sedlovskiy A, Kalmikov E (2004a) Analysis of the contents organochloride pesticides in soil of some objects of Almaty and Akmola regions. Biotechnology Theory Pract 3:99–105 (in Russian)

Nurzhanova A, Kulakow P, Niketevich L, Sedlovskiy A (2004b) Characteristics of plant species on sites contaminated by organochlorine pesticides in Almaty oblast. Biotechnology Theory Pract 4:98–110 (In Russian)

Nzengung VA, Jeffers P (2001) Sequestration, phytoreduction, and phytooxidation of halogenated organic chemicals by aquatic and terrestrial plants. Int J Phytoremediation 3:13–40

Pascal-Lorber S, Lauren F (2011) Phytoremediation techniques for pesticide contaminations. In: Lichtfouse E (ed) Alternative farming systems, biotechnology, drought stress and ecological fertilisation. Springer, New York, pp 77–105

Perkovich BS, Anderson TA, Kruger EL, Coats JR (1995) Enhanced mineralization of [^{14}C]-atrazine in *Kochia scoparia* rhizospheric soil from a pesticide-contaminated site. Pestic Sci 46:391–396

Phillips TM, Seech AG, Lee H, Trevors JT (2005) Biodegradation of hexachlorohexane (HCH) by microorganisms. Biodegradation 16:363–392

Prasad MN (2003) Phytoremediation of metal-polluted ecosystems: hype for commercialization. Russ J Plant Physiol 50:686–700

Prodina M (1960) Botanical microtechnique. High School, Moscow

Raina V, Suar M, Singh A, Prakash O, Dadhwal M, Gupta SK, Dogra C, Lawlor K, Lal S, van der Meer JR, Holliger C, Lal R (2008) Enhanced biodegradation of hexachlorocyclohexane (HCH) in contaminated soils via inoculation with *Sphingobium indicum* B90A. Biodegradation 19:27–40

Tahtadjan AL (1987) System of magnoliopsida. Nauka, Leningrad, pp 1–65, In Russian

Tsao DT (2003) Overview of phytotechnologies. Adv Biochem Eng Biotechnol 78:1–50

UNEP (2008) Report on enabling activities for the Stockholm Convention on Persistent Organic Pollutants. UNEP/ POPs/INC.5/1; IHPA estimates based on various sources: 22.11.2008

US EPA Method 8081B, Revision 2 (2007) Organochlorine pesticides by gas chromatography

US EPA Method 8250 A, Revision (2007) Organochlorine pesticides by gas chromatography

White J (2002) Differential bioavailability of field-weathered p, p′-DDE to plants of the *Cucurbita* and *Cucumis* genera. Chemosphere 49:143–152