

Discussion Paper No.552

**Adaptations of Plants to Metal/Salt Contained Environments:
Glandlar Structure and Salt Excretion**

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October 2002

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This work was supported by Monbukagakusho (Ministry of Education, Culture, Sports, Science and Technology Grant-in-Aid for Scientific Research (Project No 12372008, 2000), Japan; Polish “Kasa Mianowski “ Foundation, 2000 and (International Research & Exchange Board Program (IREX), Bureau of Educational and Cultural Affairs, USA Department of State, 2002.

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Adaptations of Plants to Metal/Salt Contained Environments: Glandlar Structure and Salt Excretion

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Abstract¹

A quantitative assessment was made of metal contamination levels and salinization of Kyzylkum Deserts territories and an analysis of plant/soil samples were investigated in relation to the natural cellular mechanism of plant adaptations to environmental agro-industrial pollutants. Preliminary results provide strong evidence that tailing sands and wet-marsh lands polluted with cadmium, copper, iron, nickel, manganese, chromium, strontium, lead, zinc, various toxic salts and organic pollutants are first colonized by plant species that develop strategies for avoidance and/or tolerance to toxic metals.

Our analyses show that the native desert plants, grown on the metalliferous and/or salinized soils tend to accumulate the highest ions concentrations in epidermal and subepidermal tissues, as well as in water bearing parenchyma, including various glandular structures of bracts/bracteoles and perianth segments. A specific adaptive defending strategy by reproductive organs occurred between the vegetative and reproductive functional systems of the resistant plants that ensure them successful sexual reproduction and seed germination.

In Chenopods like in some species of Poaceae, glandular structures are usually bicellular, comprising a basal and cap cell, and are referred to as salt glands, trichomes or microhairs. Salt secretion is considered as an adaptive strategy to regulate plant tissue ion concentration. We suggest that the different appearance of the glandular structures in some chenopods and graminous species of Kyzylkum flora is due to differences in their function connected both with accumulation of various ions and /or secretory process. The prismatic crystals secreted by epidermal salt glands and analyzed by X-ray absorption spectrometry contain primarily cations Na, K, Ca, and anions Cl, SO₄, carbonate, although other ions such as Mg, Si, and Sr also were detected. Structurally, SEM methods also revealed a high diversity in the micromorphology of epicuticular wax (epicuticular secretion) and silicon deposits that was mostly peculiar for perennial *Salsola* and gramineous species. The natural plant-cellular mechanism of salt/metal tolerance observed in this study should permit the development and testing of more informed hypotheses regarding adaptations required for colonization and survival of plants, growing under extremely harsh arid and, simultaneously metalliferous/salinized environments. Phytoremediation technology is considered a potentially valuable technique for dealing with heavy metals, which are the most difficult pollutants to remove from soils.

¹ **Key words:** metal/salts pollution, glandular morphology, crystalloids, reproductive strategy, desert plants, Kyzylkum ecosystems.

Introduction

The root causes of environmental problems in the Kyzylkum Desert often are located in agro-industrial areas. Uzbekistan has a special problem in dealing with hyper arid areas, such as the Southeast and Central Kyzylkum and the Fergana Valley, where gold, uranium mining, gas-and oil extracting and processing are concentrated. High human impact (urban, industrial and agricultural activities, handicrafts and traffic) on these territories leads to the pollution of sands and irrigated lands with pesticides, nitrates, organic pollutants and various heavy metals (Goldshtein 1997, Solodov 1998, Toderich et al 2001a, Tsukatani et al 2001).

Salinization is a second major ecological and production problem currently facing the agricultural sector in arid/semiarid areas of Central Asian countries. Recent use by the former USSR of the major rivers of Central Asia for the production of exports, including cotton, oil, and minerals, has resulted in rising water tables, waterlogging, secondary salinization (human caused) and the well known ecological disaster of the saline lands around the receding Aral Sea. It is estimated that a poor natural drainage capacity, an irrigation network with ground waterbeds, flooding, low efficiency of drainage system etc., caused the rise of mineral content of both superficial and underground waters (Kamalov 1995, Goldstein 2000, Tsukatani et al 2001, Toderich et al 2001a, b). Nowadays is crucial, but very difficult to rehabilitate and sustainable develop the salt affected and waterlogged lands that makes about 65% of the territory in lower reaches of the Syrdarya, Amudarya, and Zerafshan River Valleys.

Several authors have pointed out recently that the use of plants that hyperaccumulate heavy metals in their aerial parts could be an economically efficient method for cleaning the soils (Kiekens et al 1982, Williams et al 1994, Leblane et al 1999, Escarre et al 2000). J.M. Russeil (1992) has hypothesized that many toxic agents are natural organic chemicals encompassing a great variety of structural types. Significant progress has been made in recent years in developing native or genetically transformed plants for the bioremediation of environmental contaminants (Rugh et al 1996, Heaton 1998, Meagher et al 2001).

Initial explorations of natural plant cellular mechanisms affecting the phytoremediation of elemental and/or organic pollutants suggest great promise for the use of desert plants in large-scale environmental clean-up efforts. Factors that relate to the make-up of chemical compounds such as tannins, nitrates, metals, salts or oxalates, some of which may be toxic for plant development, have not been adequately studied with the arid flora of Central Asia. Native plants have the advantage of being highly adapted to the local climatic and edaphic contaminated conditions. Some representatives of genus *Salsola*, *Aeluropus* and *Eremopyrum* are considered an important pioneer species under metalliferous and salinised soils (Lipshitz & Waisel 1974, Banassova 1990, Naidoo 1999). It is also known that vegetative-reproductive plant systems are extremely sensitive to strong and changeable desert environments (Jordan & Kruger 1998, Toderich 2001). The morphology and structure of salt glands and mechanism of ion accumulation for Asiatic desert plants is unknown. The study of these compounds (origin, localization etc.) for Asiatic desert plants are of great interest because they often are specific to a particular plant species or genus and must therefore have been designed to serve a particular protective function. Chemical defenses also may be an important plant characteristic influencing food choice by many livestock species.

Studies of “plant functional ions/translocation system” will not only provide

important information about the soil/plant relationships, but could be an alternative approach for the analysis of natural plant ecosystems, their reaction to harsh environmental factors, and may allow predictions about vegetation spatial distribution and changes under natural and anthropogenic influences. This research is intended to contribute to the practical utilization of Asiatic desert plants for the revegetation and reclamation of metal and salt damaged lands of Kyzylkum desert ecosystems. We have focused our research on the natural metalliferous pollutants of Kyzylkum Desert because of their extreme toxicity, carcinogenicity, wide distribution and slow biodegradation under the extreme arid environments.

The main aim of the paper was to study natural soil-plant remediation systems, as well as the morphology of glandular structures in floral organs of some Asiatic desert plants, growing under metalliferous and salinized regimes whether caused by natural or anthropogenic factors.

Material and methods

The quantitative assessment of environmental impact by salinization and industrial mining pollution in the Kyzylkum deserts was conducted using geocological aerial photographs at the scale 1:500 000 (Goldstein, 1997).

The study area encloses a heterogeneous landscape comprised of sand dunes, gypsum flats, and clay and solonchaks depressions. The species studied undergo extreme, continental, arid conditions that have a limited and unreliable precipitation and a minimal annual precipitation (MAP) = 100-180 mm. The region is dominated by sandy soils with poor vegetation cover. Most of the selected sites are located inside or close to gold-uranium and gas industry complexes. At each site three cylindrical soil cores (depth 5-10 cm) were taken from within quadrats measuring 100 by 100m. Plant samples were taken from the sites that were within an area of 20 by 20 km. Each plant sample was cut into small pieces, dried at 105 °C, ached at 500 °C for at least 24 hours, mixed thoroughly, and transferred into plastic containers. After that, the various spectrometric measurements for metal content in the plant-dried materials were done using ICP-MS (Perkin Elmer-Sciex. ELAN 6000).

Samples of plant examined in this paper cover the full range of plant life forms. Many are herbs or small woody-based shrubs, although some are large shrubs 1-3m in height and a few tree-like species.

Assimilatory and generative organs of 16 species of *Salsola* (both annual and perennial), *Eremopyrum orientale* and *Aeluropus litoralis* on the different stages of their ontogenesis were studied by light and electron microscopy. Anatomical sections of bracts, perianth segments, anthers, embryo and fruits were selective stained with safranin in combination with fast green, haematoxylin or toluidine blue.

Two methods of sample preparation were used for SEM i.e. chemical fixation and freeze-drying. For chemical fixation, material was fixed, post-fixed and dehydrated as described for TEM, critical point dried (SAMDRI-780), putter-coated with gold and observed with a Philips SEM 500 (Bozolla & Russell 1991).

Different floral organs of all examined species were immersed in 3% glutaraldehyde in sodium cacodylate phosfate buffer (PH 7.2) for 3 hours prior to mounting on stubs. The material was then placed in an Edward freeze-dryer for 24h-55 °C. Specimens was coated with carbon. The salt secretions on freeze-dried leaf-like

organs (bract/bracteoles and perianth segments) were analyzed by energy dispersive X-ray microanalysis (EDX) with a JEOL JSM –T330A SEM. The elemental composition of crystalline deposits associated with salt glands with various ecological types of arid plants was determined.

Characteristics of Kyzylkum Desert Ecosystems

The Kyzylkum («the red sands») is located between two large rivers of Middle and Central Asia, the Syrdarya to the east and the Amu-Darya to the south and southwest. These two rivers flow from the massive mountain chains of the Tiyen-Shan, Pamir-Alay and Turkestan to the west and southeast respectively, and drain into the Aral Sea to the northwest (Figure 1).

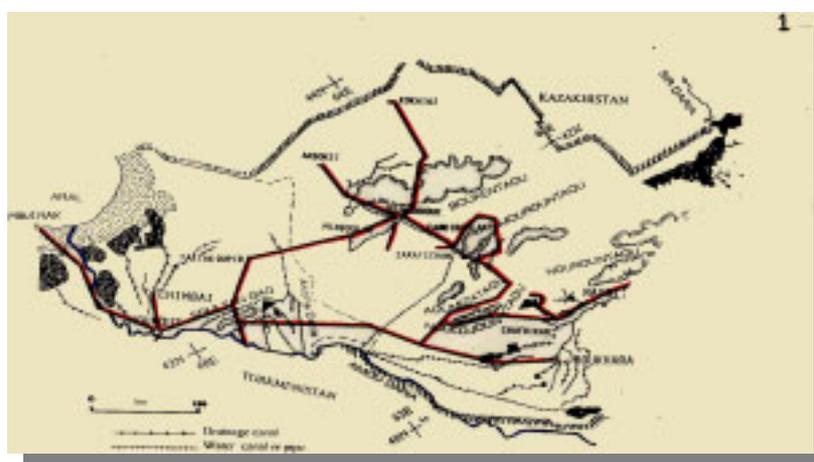


Figure 1 Map of Kyzylkum Desert on the territory of Uzbekistan

The Uzbek "Chul" are gently rolling low lands with elevation between 100 to 500 m. It encloses a heterogeneous environment comprised of sand dunes, gypsum flats, and clay and solonchaks depressions. Their climate undergoes extreme, continental, arid conditions, limited and unreliable winter precipitation, and a high level of evapotranspiration, and extreme daily, seasonal and annual fluctuations of air temperatures. As a result of these extreme conditions, it has a sparse, but diverse, vegetation cover. Most of the selected sites are located on wetlands, inside or close to gold-uranium, oil- and gas industries complex. This includes the areas affected by conventional mining and processing of uranium ores, as well as the operation of in situ leaching facilities. It was determined that pollution by heavy metals and organic pollutants is concentrated around mining and tailing (waste) deposit zones, and exclusively in and exclusively in the foothills areas of Central Kyzylkum, such as: Kul'dzhuktau, Auminzatau, Tamdutau, Dzhemtau, Aristantau and Bukantau mountain ranges. As is shown on Figure 2 highly salt impacted soils are widely distributed in the Bukhara oasis (Southeast Kyzylkum), as well as on numerous solonchak depressions: Aydarkul, Ayakagitma, Mingbulak, Beshbulak, Kulkuduk, Karakata, Karasugursk, Kukayaz. Patchy salinized lands also occur in the

technogenic industrial areas that are located between sandy-loam/clay soil formations and large sand dunes areas of the Central Kyzylkum. In the last few years there has been a tendency for a fast degradation on floodplain ecosystems of the Amudarya and Zerafshan River deltas –marginal territories of the Kyzylkum desert.

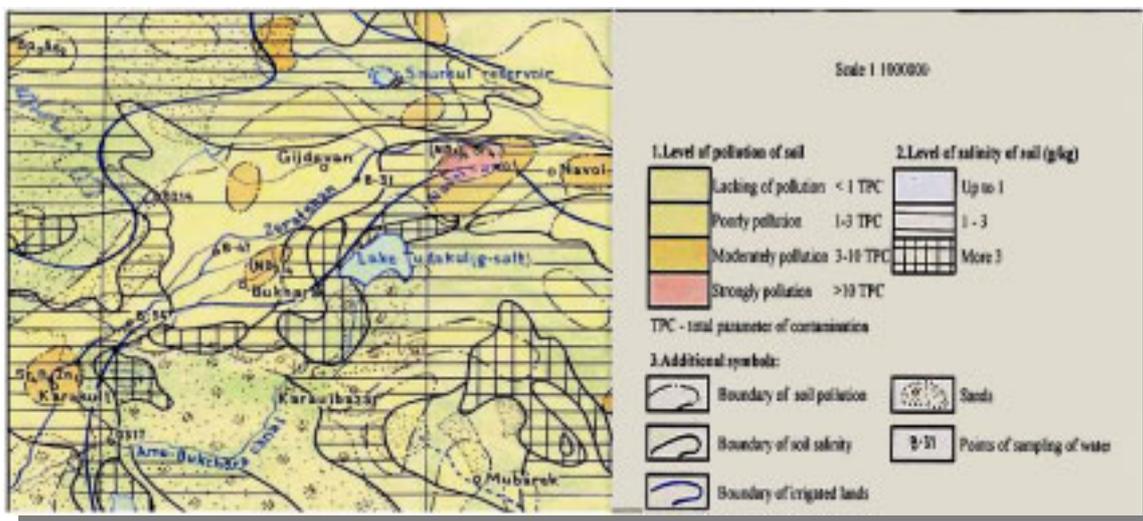


Figure 2 Extent of salinization and pollution of soils in Southeast Kyzylkum

A low productivity and high salinity (1.2-2.0 and rarely more than 3.0 % of soluble salt) characterize these soils, with a dominance of carbonates, sulfate, chloride and/or mixed types of salinisation. The predominate type of soils salinization is sulfate-hydrocarbonate-calcium. In the Central Kyzylkum, which is less salinized, the sulfate-potassium-sodium (and rarely chloric-potassium-sodium) types occur frequently. The humus content ranges from 0.5 in sandy desert and gray-brown sites to 0.7-1.2 % in the virgin and newly irrigated takyr (clay soil areas in desert and semidesert of Central Asia and South Russia. The surface of the takyr is flat, and marked by polygonal cracks in summer), widely distributed in the Southeastern Kyzylkum.

Results and discussion

Average values of trace elements composition in various soil types of the Central Kyzylkum deserts showed high levels of Hg, Cu, U, As, Zn, Mo, Ni, Sr, Co. Soils contaminated with As, Zn, Ni, Mn, Cu, Sr were found to be toxic and widely distributed in sandy Kyzylkum Desert soils. Coefficients of concentrations ($K_k = C_f/C_k$) of heavy metals exceeded 1.0.

Nickel is of natural origin and originates in rock rich in Ni and Co, particularly of Palaeozoic age. Concentrations of Ni in soils are in the ranges of 60-70 ppm. The mobility of As, Cu, Zn along with other heavy metals and their accumulation are highly facilitated both by soil chemical properties and the aridity of Kyzylkum Deserts.

It was expected that the cellular structures of plants that colonize such types of soils suffer strongly from multiple environmental stresses: high soil ionic concentrations,

impenetrable soil layers, high sun radiation, and water deficiency. Our experiments demonstrate that only a restricted number among Kyzylkum sandy/deserts plant species have the ability to establish them under high metal and salt soil concentrations and translocate a wide variety of elements. As shown from Table 1 concentrations of elements, depending of species, range in a wide variety, such as: Fe (15-4170); Zn (9.0-50.0); Pb (0.1-7.6); Ni (0.0-3.7); Cr (0.1-50.0); Sr (0.0 –793.0); or trace levels (e.g. As (0.1-1.9); Co (0.1-2.7); Th (0.1-2.5); Cd (0.1-0.18).

A survey of most of the Kyzylkum halo- and metallo-flora has revealed few species in Uzbekistan, mainly from genera *Salsola* (both annual and perennial ones), *Haloxylon*, *Halothamnus*, *Halostachys*, *Kallidium*, *Anabasis*, *Tamarix*, *Artemisia*, *Peganum*, *Zygophyllum*, *Aeluropus*, *Eremopyrum*, *Poa*, *Allysum*, *Limonium*, *Carex*, *Euphorbia*, *Frankennia*, *Lycium*, less *Astragalus* and *Smirnowia*. Specimens of the *Triticum sp. (Poaceae)*, growing on the cultivated foothill fields of the Central Kyzylkum, showed high concentrations of Fe (up to 2547ppm) in the aerial dry matter of plants. However the remarkable Zn level above 5,020 mg/kg, and deserving of being described as hyperaccumulation, was recorded for *Artemisia diffusa (Asteraceae)*. *Haloxylon aphyllum*, *Tamarix hispida*, *Artemisia diffusa*, some *Salsola* species and *Peganum harmala* demonstrated a strong tendency to translocate Sr. Besides that *Artemisia diffusa*, to a lesser extent *Tamarix hispida*, *Alyssum desertorum*, *Carex pahystylis*, *Triticum spp.* and many species of *Salsola* have a multi-element accumulation capability to Ni, Cr, Sr and Fe. Plant species exhibit differences in their ions/metals distribution characteristics (Figure 3). Representatives of genera *Salsola* maintained large concentrations of Zn in their tissues over wide ranges of soil variation and metal concentrations, indicating hyperaccumulation. Conversely, Zn extraction by *Artemisia diffusa* is relatively higher in relation to comparatively small variation of soil Zn concentrations. The relationship between the concentrations of Co in the plant tissues of *Salsola* species and soil was curvilinear, showing that this taxon is capable of accumulating large concentrations of Co across a wide range of soil concentrations.

We found that many species from various level of soil pollution recorded a high survival and normal sexual seed reproduction, but they were characterized by a low dry biomass production. They also demonstrated an insignificant phenotypic variability in the reproductive organ ontogeny. No anomalies during embryo and seed development were observed. Differences were found mostly in the morphology of seeds, level of their viability and rate of germination. This suggests that an adaptation like process occurred between the vegetative and reproductive functional systems of the resistant plants that ensure them successful sexual reproduction and seed germination.

Table 1 Average values (mg/kg) of trace elements in the aboveground dry matter of field-grown plants of Central Kyzylkum region

	Fe	Mn	Sr	Pb	Zn	Cu	Mo	Cd	V	As	Sb	Se	Ni	Co	Cr	Th	Be
<i>Alhagi pseudoalhagi</i>	<u>315</u> 280-350	<u>36.1</u> 29-43	<u>170.5</u> 85-256	<u>0.0</u> 0.0	<u>26.2</u> 17-35	<u>8.3</u> 7-10	<u>2.7</u> 2-3	<u>0.1</u> 0-0.1	<u>0.7</u> 0.5-0.9	<u>0.1</u> 0-0.2	<u>0.0</u> 0.0	<u>0.2</u> 0-0.3	<u>2.5</u> 2-3	<u>0.3</u> 0.3	<u>1.5</u> 1-3	<u>0.1</u> 0.1	<u>0.0</u> 0.0
<i>Peganum harmala</i>	<u>865</u> 15-3310	<u>38.2</u> 14-86	<u>234.3</u> 0-793	<u>0.3</u> 0-3.6	<u>24.2</u> 14-58	<u>7.2</u> 3-13	<u>2.5</u> 0-6.4	<u>0.1</u> 0-0.2	<u>2.0</u> 0-11	<u>0.1</u> 0-0.3	<u>0.0</u> 0-0.2	<u>0.5</u> 0-0.2	<u>2.0</u> 0-6	<u>0.5</u> 0.1-2.7	<u>3.2</u> 0-11	<u>0.3</u> 0-2,5	<u>0.1</u> 0-0.2
<i>Carex physodes</i>	<u>395</u> 250-580	<u>22.3</u> 19-26	<u>65.7</u> 48-104	<u>0.5</u> 0-3.2	<u>17.2</u> 11-28	<u>8.9</u> 3-21	<u>2.2</u> 0-4.6	<u>0.2</u> 0.1-0.4	<u>1.8</u> 1.4-2.5	<u>0.0</u> 0.0	<u>0.1</u> 0-0.1	<u>0.0</u> 0.0	<u>1.6</u> 0.9-2.8	<u>0.2</u> 0.2-0.3	<u>2.4</u> 0.9-4.7	<u>0.1</u> 0-0.2	<u>0.0</u> 0-0.1
<i>Poa bulbosa</i>	280	24.6	53	0.0	18.8	9.1	3.6	0.18	3.1	0.0	0.02	0.0	2.0	0.14	4.4	0.08	0.07
<i>Carex pahystyllis</i>	<u>685</u> 500-870	<u>71.5</u> 18-25	<u>57</u> 21-93	<u>0.4</u> 0-0.8	<u>16.8</u> 16-18	<u>7.6</u> 7-9	<u>2.6</u> 0-5.2	<u>0.2</u> 0.1-0.2	<u>1.5</u> 1.2-.,8	<u>0.2</u> 0-0.4	<u>0.1</u> 0-0.1	<u>0.5</u> 0.3-0.6	<u>5.3</u> 1-9	<u>0.3</u> 0.2-0.4	<u>24.6</u> 2-47	<u>0.1</u> 0.1-0.2	<u>0.1</u> 0-0.1
<i>Artemisia diffusa</i>	<u>932</u> 117-5020	<u>38.1</u> 15-128	<u>123.6</u> 13-980	<u>0.4</u> 0-7.5	<u>23.3</u> 9-72	<u>11.6</u> 3-25	<u>3.1</u> 0-9.3	<u>0.1</u> 0-0.4	<u>2.4</u> 0-15	<u>0.2</u> 0-1.9	<u>0.0</u> 0-0.5	<u>0.3</u> 0-7.6	<u>3.3</u> 0.-12	<u>0.5</u> 0.1-1.9	<u>3.7</u> 0.5-14	<u>0.3</u> 0-1.2	<u>0.1</u> 0-0.4
<i>Triticum sp.</i>	<u>2547</u> 440-4650	<u>62.7</u> 10-150	<u>82.3</u> 27-135	<u>2.4</u> 0-7	<u>25.3</u> 16-38	<u>15.3</u> 6-25	<u>1.3</u> 0-3.6	<u>0.2</u> 0.1-0.4	<u>7.1</u> 1-14	<u>0.1</u> 0-0.6	<u>0.2</u> 0.1-0.5	<u>0.2</u> 0-0.5	<u>5.6</u> 1-13	<u>1.1</u> 0.3-2.2	<u>20.6</u> 2-50	<u>0.8</u> 0.1-1.5	<u>0.1</u> 0-0.3
<i>Haloxylon aphyllum</i>	<u>454</u> 100-2600	<u>42.0</u> 18-82	<u>146.3</u> 53-1041	<u>0.3</u> 0-4	<u>19.2</u> 11-41	<u>5.8</u> 2-13	<u>2.7</u> 0-7.1	<u>0.1</u> 0-0.2	<u>0.6</u> 0-3.4	<u>0.1</u> 0-0.4	<u>0.0</u> 0-0.2	<u>0.2</u> 0-1	<u>3.6</u> 0-24	<u>0.2</u> 0.1-1.1	<u>2.1</u> 0.3-23	<u>0.1</u> 0-0.8	<u>0.0</u> 0-0.1
<i>Salsola L.</i>	<u>569</u> 160-1880	<u>41.6</u> 16-93	<u>153.6</u> 21-508	<u>0.5</u> 0-7.6	<u>20.8</u> 8-50	<u>6.7</u> 3-12	<u>1.6</u> 0-5.6	<u>0.1</u> 0-0.5	<u>2.0</u> 0-12	<u>0.1</u> 0-0.5	<u>0.0</u> 0-0.2	<u>0.9</u> 0-13.6	<u>1.8</u> 0-4	<u>0.4</u> 0.1-0.8	<u>2.0</u> 0.5-5.5	<u>0.1</u> 0-0.7	<u>0.1</u> 0-0.2
<i>Tamarix hispida</i>	2960	74.0	264	0.0	20.1	6.4	0.9	0.09	8.1	0.0	0.09	0.2	5.7	3.08	8.5	0.87	0.38
<i>Ferula assa-foetida</i>	<u>755</u> 510-1100	<u>32.2</u> 25-50	<u>147</u> 116-166	<u>0.1</u> 0-0.5	<u>21.3</u> 17-33	<u>21.4</u> 9-54	<u>1.5</u> 1-2	<u>0.1</u> 0-0.4	<u>1.8</u> 1.1-2.6	<u>0.0</u> 0.0	<u>0.1</u> 0-0.2	<u>0.0</u> 0.0	<u>2.1</u> 1.2-3.7	<u>0.3</u> 0.2-0.4	<u>3.2</u> 1.2-3.6	<u>0.2</u> 0.2-0.3	<u>0.0</u> 0.0

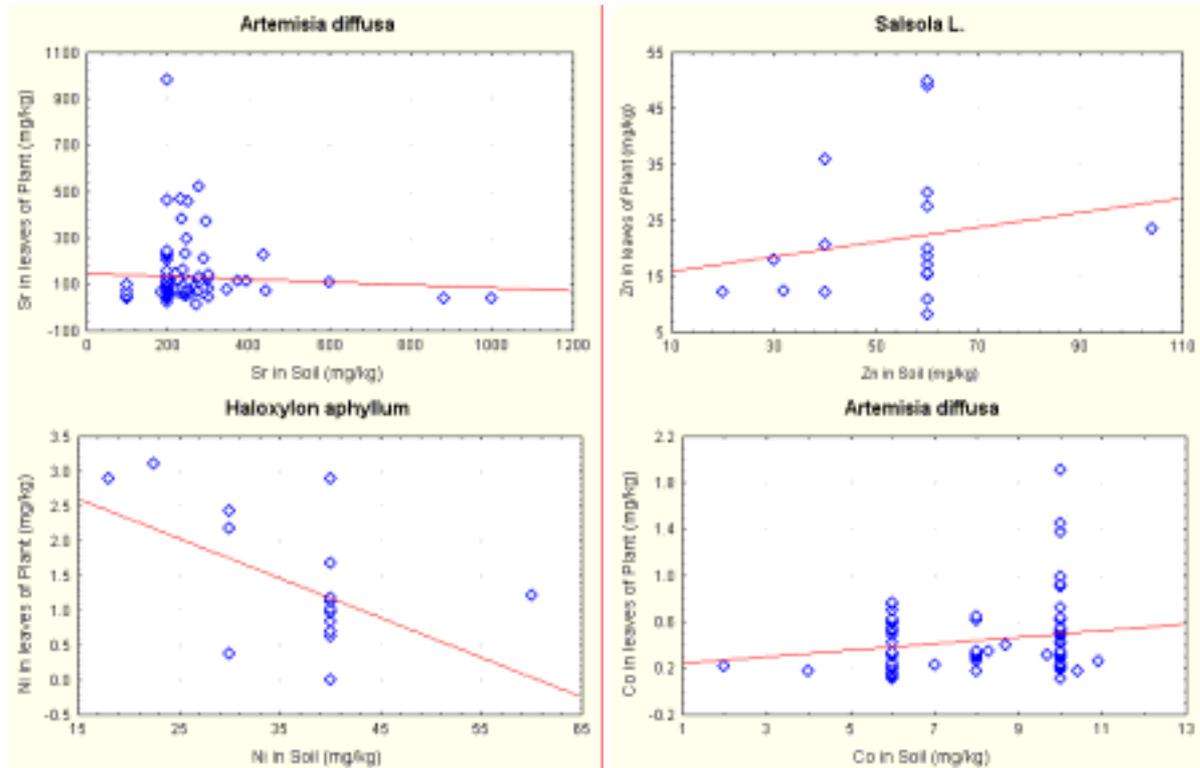


Figure 3 Ions/metals distribution characteristics in various desert taxa

Our analyses show that these desert plants, grown on metalliferous or salinized soils, tend to accumulate the highest ion concentrations in epidermal and subepidermal tissues, including various glandular structures of bracts/bracteoles and perianth segments. Salt glands that are recognized as structures of varying degree of specialization, actively involved in the elimination of solute, mineral elements on the surface of the vegetative organs are very common for Asiatic desert plants. Excretion occurs predominantly on the adaxial surface and is uniformly localized along the lateral walls of the grooves. Salt glands vary somewhat in different genera in that they may be sunken, semi-sunken or located above the epidermis as in the majority of investigated chenopods and gramineous species. In chenopods and in gramineous species glandular structures are usually bicellular, comprising a basal and cap cell. Slight variations in morphology of the basal and cap cells of glandular hairs were described mostly with annual species of genus *Salsola*, *Aeluropus litoralis* and *Eremopyrum orientale*, which appear to be related to their efficiency of salt secretion. The analogy was described for *Cynadon* and *Distichlis* species (Thomson et al 1988) where large flask-shaped, sunken basal cells and dome-shaped cap cells secrete salts more efficiently than trichome-like glands with narrow basal and cap cells as in *Sorghum halapense* (McWhorter et al 1995) and *Sporobolus virginicus* (Naidoo 1998).

We frequently observed epidermal vesicles and papillae in desert species. As seen on the Figure 4., such type of epidermal structures have a large, bladder cell attached to a stalk composed of one or more cells that in turn is attached to an epidermal cell. Comparative study of two annual taxonomically close related *Salsola* species from steppe soils of Europe (Poland) and Kyzylkum metalliferous/salinized sands revealed that salt secretion become prominent and salt glandular structures are formed abundantly only when plants are exposed to high contaminated environments. Under such conditions an evident increasing of succulent bracts was consistency noted with Kyzylkum chenopods.

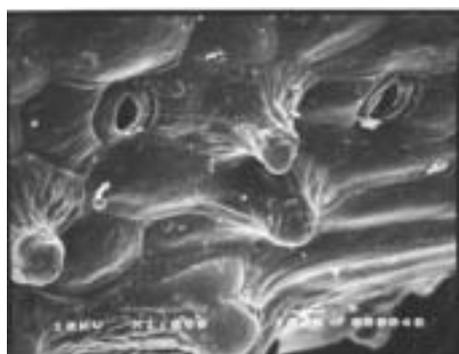


Figure 4 The morphology of vesicular- and short peltate trichomes on bracts of *Salsola pestifer* (Bukhara ecotype).

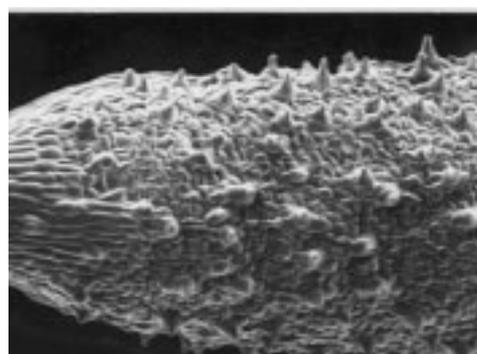


Figure 5 Epidermal surface views of mature bracts of *Salsola pestifer* (Bukhara ecotype). Glandular structures have a strong localization, especially on adaxial side, which is mostly exposed to environmental impact. X 750

We suggest that the vesiculate hairs of some Asiatic annual *Salsola* are considerably involving in cellular salt secretion mechanisms. Although in the opinion of Luttge 1971, this might not be taken strictly as a secretor process, these trichomes are considered salt glands review since their function is obviously a specialized mechanism for the removal salt from the leaves. The emission of salt in these vesiculated hairs is apparently the result of the rupturing and collapses of bladder cells (Luttge 1971). The presence of papillas on the epidermal cells as was described for *S. praecox*, *S. iberica* and *S. pestifer* (Figure 5), their thick outer walls, cuticle and submerged stomata seemingly protect assimilatory organs against excess transpiration. Annual C4- herbaceous species, known in practice of pasture economy of Uzbekistan as “solyanki” differ by salt-glands/trichomes morphology (shape of their head: mainly clavate or capitate or also in the number of constituent cells composing their stalk) and its density on the epidermal surface. Variation in salt glands /trichomes density is believed to be mainly the effect of stress/desert environmental factors and even herbivore pressures (Johnson, 1975). These parameters potentially could be used as discriminating characters between different ecological halophytes groups. For instance the *Climacoptera* complex has a unicellular non-glandular trichomes or hairs possessing a smooth or micropapillate (warted surface), while the bract/bracteoles surface of many

dry/sclerified *Salsola* species has an indulating epidermal surface with numerous salt glandular structures and tall adaxial ridges alternating with deep grooves. On the ridges of annual *Salsola* species we found various papillae and prickles hairs, as well as secreted salts, which appear as crystals. Crystalline deposits were more abundant on the adaxial organ surface because of the higher gland frequency (Figure 6, 7).

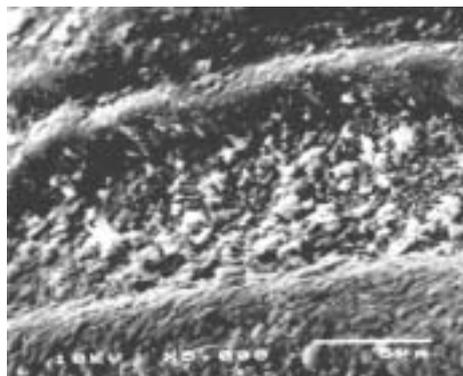


Figure 6 The adaxial surface of bracts of *S. iberica* with ridges and salts which appear as crystals.

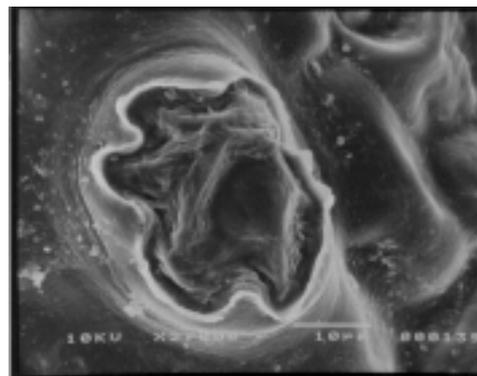


Figure 7 Scanning micrographs showing the patterns of crystalloid structure in the bract tissues of *S. orientalis*.

Salt glands (bladder cells –structural organization) usually are globose or club-shaped and readily distinguishable from unicellular papillae and sharp-pointed prickles. (Figure 8-11). An ornamented, porous cuticle overlies the epidermis. The cuticle is distinctly thicker over the area that adjoins basal and epidermal cells than over the cap or other parts of the epidermis. The cuticle is separated from the outer cap cell wall, resulting in the formation of a salt collecting chamber or cuticular cavity. The cuticular cavity we noted for species of *Salsola* (both annual and perennial), *Aeluropus litoralis* and *Eremopyrum orientale* is similar to those in *Spartina*, *Cynadon* and *Distichlis* (Thomson 1975) and probably represents a temporary collecting compartment where secreted salts accumulate prior to elimination from the leaf. The ions seems to be compartmentalized in small vacuoles and transported to the cuticular cavity, prior to exclusion from the vegetative and reproductive organs either through cuticular pores or by rupture of the cuticle (Yordoan 1998, Naidoo 1998).

An unusual type of salt glandular structure was described for *Salsola carinata*. As shown on the Figure 11, the terminal cell(s) is always end bluntly. On top of the stalk cell, extremely thin-walled cells form a single originally ornamented ring, while the thick cuticle of the stalk cell remains as a cylindrical scar.



Figure 8 Salt gland of *Salsola paulsenii* comprising flask-shaped basal cell, dome-shaped cap cell and raised cuticular chamber



Figure 9 Micromorphology of glandular hairs of *Salsola sclerantha* and wax-epicuticular inclusions partially surrounding it.



Figure 10 SEM micrograph showing surface features and morphology of non-glandular, unicellular hair of bracts in *Cimacoptera lanata*.



Figure 11 Untypical morphology of salt gland, occurring on the epidermal bract's surface of *Salsola leptoclada* (Central Kyzylkum ecotype).

Cross-sections of bracts and bracteoles of many *Salsola* species shows that different tissues carry out water and salt-accumulating functions: swollen epidermal cells (in all species), large-celled hypodermis and water bearing parenchyma (Figure 12). Their size, shape or density can be recognized by the location and deposits of salt/ions into specific (salt- storage) cells (Figure 13). The displacement of salt ions in the flower organs of some *Salsola* species by means of fluorescent microscopy reveals an abundance of

mineral ions in the tissues of sterile of flower like sepal's elements (Figure 14) or anther connective cells (Figure 15). However ions dislocation has never been observed in male- and female gametophytes or in embryos.



Figure 12 Cross-section of succulent bract of *Salsola praecox*; central part is occupied by 3-4 layers of water – storing parenchyma cells with small salt crystals. 10 X 60 (1 μ k)

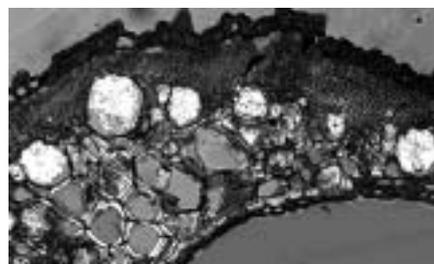


Figure 13 Anatomy of bract tissue in *Salsola arbusculiformis*. Different types of crystals in the subepidermal salt-storage cells. 10 X 60 (3.0 μ k)



Figure 14 Cross section of anther in *Salsola arbuscula*. The salt ions location in pollen grains (male gametophyte) is absent. 10 X 60 (1 μ k)

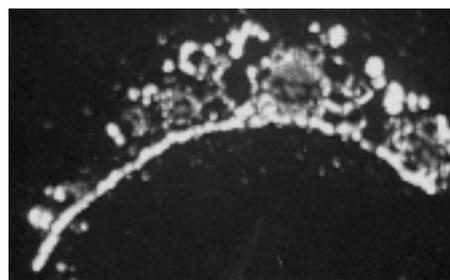


Figure 15 The fluorescent microscopy image of bract of *S. arbusculiformis* with the location of salt/ions in it. 10X 60 (1 μ k)

Occurrence of calcium oxalate crystals in leaves and seed coats of poisonous plants was described by Fuller & McClintock, 1986. It was suggested that the concentrating of oxalate crystals was almost absent in the root and stems. The presence of crystals in the outer covering of seeds may play a role in changing soil pH, thereby providing a more favorable condition for plant's survival.

Structurally, SEM methods revealed a high diversity in the micromorphology of epicuticular wax (epicuticular secretion), mostly occurring as specific crystalloids on the plant surface of these desert plants. Cuticular wax partially covering mature prickle-hairs, papillas and long cells of outer epidermis of bracts/bracteoles of some perennial *Salsola* species is shown on Figures 9, 16. Unfortunately the nature and molecular organization of such wax deposits is still unknown for desert plants. Therefore we shall use the term crystalloids (epicuticular wax crystalloids) proposed by Barthlott et al 1998, which

recognize this uncertainty. There is also evidence in the literature that chemically these waxes consist of alkanes, long-chain alcohols, ketones, and esters of long-chain fatty acids, cyclic compounds like phytosterols, pentacyclic triterpenoids and epicuticular flavonoids (Barthlott 1994, Barthlott et al.1998). However there are still contradictory opinions concerning waxes deposition. Hall et al 1965 suggested that waxes could be exuded to the outer cuticular surface through pores, while Hallam (1970) and Mahllberg (1991) suggested excretion through lamellate regions onto the cuticle. Glandular trichomes in such case enhance a plant capacity to accumulate large quantities of volatile components and transport them to the cuticular surface for vaporization from the gland surface.



Figure 16 Scanning micrograph of *Salsola orientalis* bract epidermal surface with various salt crystaloids (or epicuticular inclusions) on it.

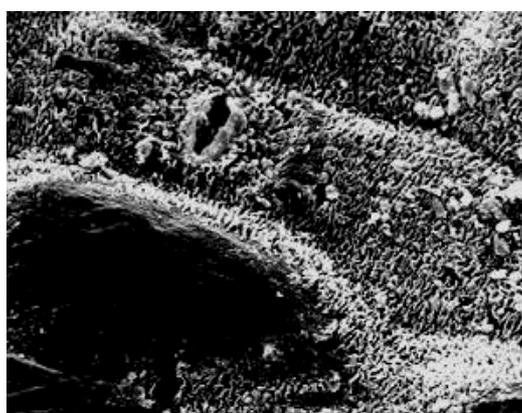


Figure 17 Silicon X-ray distribution image of mature inflorescence bracts in *Eremopyrum orientale* (*Poaceae*) X 3000

A comparative developmental study of floral organs of various chenopods and gramineous species revealed that Si accumulation was greatest on the adaxial trichomes of inflorescence organs of *Eremopyrum orientale*, *Bromus tectorum* and *Aeluropus litoralis*, collected from highest contaminated lands from the Bukhara oasis. The localization of small siliceous particles on the inflorescence bracts of *Eremopyrum orientale* that are concentrated mostly on the surface of epidermis around stomata is shown on the Figure 17. Crystalloid types in *Salsola* taxons are characterized by uniformly distributed small irregular-shaped platelets; occasionally they have also a parallel orientation around the stomata. In some chenopods species platelets occur in clusters too. The similar silicification process associated with trichomes and other epidermal structures of the inflorescence bracts was described for *Phalaris canariensis* L (*Poaceae*). There is an opinion that the silicification may be synchronized with the deposition of wall substances, such as lignin, suberin and phenols (Sangster & Wynn Parry 1981). Silicon deposition patterns and localization in bracts has been described for different groups of flowering plants (Sangster et al 1983). Siliceous hairs and particles have been suggested as possible causative factors of human oesophageal cancer (Hodson et al 1983).

Electron microscopic X-ray analysis of salt glands secretion products with different representatives of *Salsola* has revealed the localization of variety of mineral elements and ions. Prismatic crystals secreted by glands primarily contain cations Na, K, Ca, and anions Cl, SO₄, carbonate, although other ions such as Mg, Si, and Sr also were detected. We believe that our findings require further studies on a wider range of plant material in respect to structural and genetic variation and their relation to bioremediation of contaminated desert ecosystems.

Conclusion

These results show that tailing sandy and saline soils, contaminated with Cd, Sr, Cu, Fe, Ni, Mn, Cr, Pb, Zn, various toxic salts and organic pollutants are colonized by plant species that develop strategies for avoidance and/or tolerance to metal toxins. One possible avoidance strategy is for plants to prevent the uptake of potentially toxic metals, especially into the reproductive organs like pollen grain (male gametophyte) and embryo. This mechanism still is not strong analyzed in arid vascular plants, although tolerant plants seem to restrict salts and metal uptake to varying degrees. It was found that salt (minerals and ions)-accumulating glands are mostly common in families *Poaceae*, *Tamaricaceae*, *Chenopodiaceae*, and *Frankeniaceae*, but only occurred in a few scattered species from *Plumbaginaceae*, *Zygophyllaceae*, *Fabaceae*, *Lamiaceae*. Many species of these families are known to have glandular structures, but in many instances further investigations are needed to determine their secretion products.

Using morphological characters, mainly related to epidermal structures (by SEM analysis), we find that the *Salsola* species complex could in fact be divided into two groups: species with salt-producing trichomes/hairs and salt-accumulating (with specific salt/storage cells) plants. This is an indication that different mechanisms and strategies for the sequestration and regulation of the salt ion concentration in the plant tissues are operated in the stem and leaf succulent halophytes and in the recreteo- and pseudohalophytes of the Kyzylkum flora. The ability of some desert chenopods to accumulate significant amounts of nitrates and/or oxalates has been noted in the literature (Baslavskaja 1946, Kingsbury 1964, Everist 1974, Erejepov 1978, Fuller & McClintock 1986, Judd & Ferguson 1999, Butnik, 2001). The spiny leaves or fruits or chemical constituents of them are known to cause skin irritation of animals (Mitchell & Rook 1979).

In addition, the natural plant-cellular mechanism of salt/metal removal and tolerance provided by this study should permit the development and testing of more informed hypotheses regarding adaptations required for colonization and survival of plants, growing under extremely harsh and simultaneously contaminated desert environments.

The existence of great diversity in photosynthetic pathways of Asiatic *Salsola* species, as well as in their CO₂ assimilation organs anatomy and biochemical features also is evidence related to plant's growth, survival, and reproduction in these continental deserts (Butnik et al 1991, Pyankov et al 2001, 2002).

It is worth noting here that the multicellular trichomes of vegetative sterile elements of floral bracts, bracteoles and perianth segments of some chenopods and graminous plants are related to salt and heavy metal removal. In some cases, it has been

established that a high concentration of various ions accumulates in the vacuole of bladder trichomes terminal cells. This process is related probably by the rupture of cuticle or cell walls since collapsed cells give the characteristic mealy appearance of the epidermis in many *Chenopodiaceae* species (Tomson 1975, Carolin 1983). There are two types of glandular trichomes (salt glands) found by us in *Salsola* species that contradict the literature data concerns the absence of salts glands in chenopods (Carolin 1983). It may be that they are not strictly homologous, particularly since both occur in annual *Salsola* species. We suggest that the different appearance of terminal cells by these two types is due to differences in function connecting both with the accumulation of various ions and /or secretory processes. A comparative morphology study of closely related annual *Salsola* species from highly contaminated desert soils (Kyzylkum) and unpolluted steppe soils (Europe) shows an increase of succulent bracts/perianth segments consistent with Kyzylkum chenopods. Epidermal vesicles were rarely recorded here. The prickles, as single celled hairs with relatively thin cellulose walls, and thick cuticles, as in some annual chenopods, may represent the final stage in the reduction of uniseriate hairs (type 3 and 4 according to R.C. Carolin 1983 classification). We are inclined to consider various morphological types of hairs described mostly for *Salsola* species as part of the same transformation series. Probably they perform different functions. However little is known about the origin and significance of such kind of transformation, especially when they occur on the same plant.

The use of heavy- metal tolerant flora to reclaim soils could represent both a practical and economically viable alternative strategy. Halophytes and simultaneously metal tolerant arid/semiarid plants may be used for phytoremediation of areas contaminated with toxic salts and heavy metals. The plants that were found growing well in natural metalliferous and/or high saline sandy habitats we include as metal tolerant and/or facultative metallohalophytes. We suggest that for bioremediation purposes there should be interest in any species that consistently has a metal/salt removal potential. Since several "hyperaccumulators" are characterized by small biomass production, the use of selected metallohalophytes species, as phytoremediators capable of accumulating high amounts of toxic ions should be considered.

In addition to the use of local floral resources for land phytoremediation, the introduction of new succulent halophyte species with high adaptability to severe waterlogging for forming protective water-saving mechanisms is of interest. The cultivation of halophytes (C3 and C4 plants) both native and introduced may limit long-distance salt spreading and improve the vitality and growth conditions for local species, when cultivated in combination plantations. And since stress conditions frequently trigger defense mechanisms based on the production of specific biological active metabolites of pharmaceutical or industrial importance, halo-metallophytes of the South part of Aral Sea Basin could constitute a valuable source of cash compounds. These characteristics may offer a new and valuable source of income to local populations and favor the movement from crowded impoverished towns to prosperous rural communities.

Acknowledgment:

This work was supported by Monbusho Grant-in-Aid for Scientific Research (Project No 12372008, 2000), Japan; Polish “Kasa Mianowski “ Foundation, 2000 and (International Research & Exchange Board Program (IREX), Bureau of Educational and Cultural Affairs, USA Department of State, 2002.

We would like to acknowledge to James A. Young (USDA Department of Agriculture, Reno, Nevada, USA) for his review and valuable comments.

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