Chapter 5
The Use of Higher Plants in Biomonitoring and Environmental Bioremediation

Svetlana Vladimirovna Gorelova and Marina Vladimirovna Frontasyeva

Abstract This chapter provides basic information on the use of higher plants for biomonitoring and bioremediation in the world. It contains a large amount of material of the authors' own research on the possibility of using woody plants for biomonitoring and phytoremediation of environment anthropogenic pollution with heavy metals. The species of woody plants are revealed, which are recommended for use in biomonitoring of anthropogenic pollution of the environment in temperate latitudes (the study of biogeochemical parameters of leaves): *Acer platanoides*, *Aesculus hippocastanum*, *Betula pendula*, *Cotoneaster lucidus*, *Populus nigra*, and *Salix fragilis*. The following species are recommended for phytoremediation of soils from heavy metals: *Betula pendula*, *Cotoneaster lucidus*, *Syringa vulgaris*, *Sorbus aucuparia*, *Philadelphus coronarius*, and *Larix sibirica*. The species of woody plants—bioindicators of air and soil pollution by heavy metals—are revealed. The chapter also shows the significance of the statistical analysis for the detection of the main element pollutants of the environment.

Keywords Biomonitoring • Bioremediation • Heavy metals • Woody plants • Bioaccumulation • Soil and air pollution • Statistical analysis

---

S.V. Gorelova, Ph.D. (✉)
Institute of Advanced Training and Professional Retraining of Education Employees
of Tula region, Lenina Street, Tula 300041, Russia
e-mail: salix35@gmail.com

M.V. Frontasyeva, Ph.D.
Joint Institute for Nuclear Research, Frank Laboratory of Neutron Physics,
Sector of Neutron Activation Analysis and Applied Research,
Str. Joliot-Curie, 6, Dubna, Moscow Region 149080, Moscow, Russia
e-mail: marina@nf.jinr.ru

A.A. Ansari et al. (eds.), *Phytoremediation*, DOI 10.1007/978-3-319-52381-1_5

5.1 The Use of Higher Plants for Biomonitoring (Basic Information)

Biomonitoring is a system of control and obtaining quantitative characteristics of biological objects (biomonitors) in time for the assessment of the environmental changes. The main objective of biomonitoring is to prevent the adverse effects of sequences of the environmental changes and forecasting of developments of events at the level of individual populations as well as of biogeocenosis and the biosphere as a whole.

Monitoring can be carried out at different levels: molecular, tissue, organ, organism, population, species, ecosystem, and biosphere. Depending on the venue, it can be local, regional, national, and international. It may also vary according to the objects of research and may define the parameters, being passive (carried out directly in the wild nature) or active (requires setting of the experiment by a researcher) [1–5].

To conduct biomonitoring, the major role belongs by the choice of the environmental monitors (markers) of the environmental state, the development of uniform methods of sampling, sample preparation, and the proper selection of analytical methods for different types of contaminants. Due to the fact that the spectrum of the environmental pollutants comprises more than 400,000 items, use of chemical methods of analysis only is too costly, and it does not allow to get the whole picture of their cumulative impacts on biota; so it is more cheaper to apply methods of bioindication, biological testing, and biomonitoring. However, none of biological object may be a universal indicator or monitor sensitive to various substances to the same degree.

Basic requirements for plant biomonitors are summarized as follows [2, 3]:

1. Widespread and long vegetation period and high degree of bioaccumulation of elements of the environment (passive biomonitoring), the ability to good growth in standardized conditions (active biomonitoring)
2. A clearly marked and reproducible response to certain changes in the environment and bioaccumulation of toxic elements in an amount reflecting the situation in the environment
3. High sensitivity to pollutants (diagnosis effect at low levels of contamination)

There are no universal biomonitors that meet the requirements with respect to all possible contaminants; therefore, an important task for biomonitoring is the selection of species that can be used for biomonitoring of various parameters of the environment.

Markers for biomonitoring may be molecular mechanisms: the study of the structure of DNA changes, the genetic response, and synthesis of substances [6]. Biomonitoring can be carried out at a biochemical and physiological level: determination of the content of low molecular antioxidants, involved in detoxification mechanisms when the radicals produced under stress. Such antioxidants are ascorbic acid, glutathione and proline [7–13]. The stress level can be determined by the change of activity of antioxidant enzymes superoxide dismutase, peroxidases, catalase, and glutathione reductase [14–18]. However, these plant reactions are not always specific to certain toxicants and depend on the species of the plant [19, 20].

The most commonly used is an identification sign of quantification of chlorophylls and carotenoids in plants [21–23], as well as their ratio and the response of the light phase of photosynthesis [24–26]. At the level of organelles, membrane structure, chloroplasts, and mitochondria (transmission electron microscopy, TEM) are known [27]. When studying the plants at the tissue level, histochemical methods are applied using dyes that are specific to a particular metal, which helps to determine the localization in the tissue and way of their movement and accumulation in the plant [28, 29]. As biomarkers in model experiments on determination of the effects of various concentrations of toxic substances in the environment on the plant, individual organs of plants can be used, where the biomass growth (shoots), parameters such as germination and vigor (seeds), the root test, and definition of tolerance index are studied [30–33]. In passive monitoring at the organ level, the development and percentage of leaf necrosis and chlorosis, development of deformation of shoots and leaves, and modifications of the leaf blade (the appearance of the blades in simple leaves, the absence of leaf share, threadlike leaves, etc.) are determined. Besides, one can determine the leaf square, the degree of xeromorphism, and determine the percentage of dead shoots and dry crown of trees [34, 35]. At the organism level, vitality of species in the altered environmental conditions is determined [21, 36, 37]. However, when it comes to polymetallic pollution of the environment with heavy metals and metalloids, most significant is determination of elemental (biogeochemical) composition of plants and plant organs, which may reflect the situation in the environment [36], if the plant is an *indicator* [37–40]: it adsorbs and bioaccumulates metals in the process of growth, develops mechanisms of resistance to toxic elements, and does not belong to *excluders* (which exclude) or bioaccumulators in accordance with the classification proposed by AJM Baker [41, 42].

From the plant physiology point of view, biomarker of the pollution stress effect at the level of phytocoenosis, to some extent could be chlorophyll fluorescence [24, 25]. For biomonitoring of ecosystems, geobotanical methods are also applied: analysis of the number and types of species and their vitality, crown density, and density of herbaceous (or moss-lichen) cover and the analysis of the presence of anthropogenic weeds in phytocenosis, which makes it possible to conclude about the degree of digression of the community.

According to their response to the content of toxic components in the environment, bioindicators and biomonitors may be sensitive (respond to the impact of a significant deviation from the norm) or bioaccumulative (feedback manifests itself gradually, and pollutant accumulates in the body or individual organs and tissues) [1–3].

Most often to biomonitor atmospheric deposition the higher spore plants – mosses – are used. The idea of using terrestrial mosses for the analysis of atmospheric deposition of heavy metals has been proposed in the late 1960s of the twentieth century by Rühling and Tyler [43–45]. It is based on features of moss anatomic structure and physiology. The leaves of moss are composed of 1–3 layers of cells, they lack cuticles on the leaves preventing the penetration of pollutants, they have no roots, and they readily absorb water and nutrients from wet and dry deposition by rhizoids.

Mosses effectively accumulate heavy metals and other compounds due to the large specific surface area and slow growth. As a passive biomonitor in most cases, they help to identify the impact of pollutants at the ecosystem level. Ideas of moss monitoring in Europe have been developed by Rühling et al. [46, 47], Steinnes [48, 49], Steinnes and Andersson [50], Steinnes et al. [51], Steinnes and Frontasyeva [52], Rühling and Steinnes [53], Berg and Steines [54], Schröder et al. [55], and Harmens et al. [56–60].

Since the 1970s, in the Scandinavian countries, and in the last 20 years in the Eastern, Central, and Western Europe, passive briomonitoring receives support of targeted state grants and programs, and it is held regularly every 5 years in the framework of the UN Convention on Long-Range Transboundary Air Pollution (LRTAP) [53, 58, 60–64]. Coordination of moss biomonitoring in Europe, Russia, and Asia is carried out through the United Nations program (UNECE ICP Vegetation).

Based on the monitoring results, the atlases of atmospheric deposition of pollutants are edited and published, which allow estimating the cross-border transfer of elements, reveal sources of pollution and their impact on the environment, as well as trace the retrospective distribution of elements in the atmosphere [58, 60, 61, 65].

In Russia, conducting biomonitoring first started in the northwestern regions: Leningrad region [66, 67], Kola Peninsula, and Karelia [54, 68]. Since the late 1990s of the twentieth century, biomonitoring was carried out on the basis of the analytical complex of the Joint Institute for Nuclear Research for a number of central regions of Russia: one-time study conducted in Tula region [69, 70], Tver, Kostroma, part of Moscow and Ivanovo regions [71–74], Ural [75, 76], Udmurtia [77], as well as Kaliningrad region [78–82].

In 2014 the coordination of the moss surveys in the UNECE ICP Vegetation has been transferred from the UK to Russia, Joint Institute for Nuclear Research (Dubna, Moscow Region) to M. V. Frontasyeva, so far JINR has direct access to the member-states in which the UNECE ICP Vegetation is interested in the Caucasus region and Asia: Azerbaijan, Georgia, Kazakhstan, Mongolia, Vietnam, and Moldova in the southern east. Currently, the study area of atmospheric deposition by passive biomonitoring greatly increased, and GIS mapping and transport models build on the data submitted by teams from different countries; it will be possible to make more global conclusions on the transboundary transport of substances (2015–2016 moss survey) and to create a database on the content of elements in mosses on a global scale, which can be replenished in the future [83]. In addition to atmospheric deposition of heavy metals, this method also allows evaluating the contamination of nitrogen, persistent organic pollutants (POPs), and radionuclides [58, 59, 83–87].

Besides higher spore plants for biomonitoring, woody and herbaceous plants of genera *Gimnospermae* and *Angiospermae* can be used. They reflect the state of soil, air and water may due to change of their biochemical, physiological, and morphological parameters and their ability to bioaccumulate the toxic elements from the environment [21–23, 34, 35, 88–105].

Using the higher seed plants for biomonitoring purposes has advantages over the use of spore plants: they are easily identified (e.g., than mosses) and grow in urban ecosystems, and some of them have extensive habitat areals and can be used for the diagnostics of transboundary transport of elements between countries and continents.

Table 5.1 Higher plants for bioindication and biomonitoring of the environment (Applied Ecobiotechnology [106]; Gorelova [107]; Gorelova et al. [97, 104, 105])

Species	Used in bioindication, symptoms	Used in biomonitoring	Substance to which the given type reacts
Taráxacum officinále	+, necrosis and chlorosis of leaves, the change of physiological parameters (photosynthetic pigments content of low molecular weight antioxidants)	+, accumulation of heavy metals in the body depending on the degree of pollution	Heavy metals, ozone
Gladiolus gandavensis, *Tulipa gesneriana*, *Iris germanica*, *Petroselinum crispum*	+, regional and apical necrosis, accumulation of fluorine in the dry matter	–	HF
Urtica urens	+, necrosis strips on the underside of leaves	–	Peroxyacetyl
Nicotiana tabacum, *Spinacia oleracea*, *Glycine max*, *Trifolium pratense*, *Trifolium angustifolium* and subsp.	+, necrotic changes in leaves (spots), pink spots on the leaves (reaction on pollution by ozone), interveinal necrosis (reaction on nitrogen oxides)	+	O_3, NO_2
Poa annua	+, necrosis strips on the leaves	+	NO_3^-
Medicago sativa, *Fagopyrum esculentum*, *Plantago major*, *Pisum sativum*, *Trifolium incarnatum*, *Pinus sylvestris*, *Quercus* subsp., *Platanus* subsp., *Populus* subsp., *Acer* subsp., *Fagus sylvatica*, *Fraxinus excelsior*	+, interveinal point and extensive necrosis and chlorosis. Marginal necrosis and chlorosis, early death of needles and dry crown dieback (*Pinus*)	+	SO_2
Spinacia oleracea, *Phaseolus vulgaris*, *Latuca sativa*	+, chlorosis, change the structure of chloroplasts, photosynthesis violation		Cl_2
Lepidum sativum	+, reduction of biomass, germination and growth energy, dying roots		NaCl, heavy metals
Pleurozium schreberi, *Hylocomium splendens*, *Polytrichum* sp., *Brachythecium* sp., *Hypnum cupressiforme*, *Sphagnum* subsp.		+, accumulation of atmospheric deposition	Radionuclides Sr^{90}, Cs^{137}, K^{40}

Species	Used in bioindication, symptoms	Used in biomonitoring	Substance to which the given type reacts
Pleurozium schreberi, *Hylocomium splendens*, *Polytrichum* subsp., *Brachythecium* subsp., *Hypnum cupressiforme*, *Sphagnum* subsp.	+, necrosis at high concentrations of heavy metals in the environment	+, accumulation in organs from atmospheric deposition	Heavy metals and metalloids
Fagus sylvatica, *Ailanthus altissima*, *Robinia pseudoacacia*, *Picea abies*	needle chlorosis, the appearance of dead branches in the crown	+, accumulation of toxic elements in leaves	Heavy metal
Aesculus hippocastanum	+, regional and interveinal necrosis	+, accumulation of Cu, Ni, Pb. Reduction of amount of photosynthetic pigments, decrease of ascorbic acid content, increased synthesis of phenolic compounds	Heavy metals
Betula pendula	+, asymmetry of leaves, necrosis	+, Mn, Ni, Zn, Cd, Pb, reduction of the amount of photosynthetic pigments, decreased ascorbic acid content, synthesis of phenolic compounds	Heavy metals
Populus nigra	+, decrease in vitality, necrosis of leaves, early defoliation, dry crowns	+, Fe, Ni, Zn, Cd, Pb, reduction of the amount of photosynthetic pigments, decrease of ascorbic acid content, synthesis of phenolic compounds	Heavy metal
Tilia cordata	+, regional and focal necrosis	+, reduction of photosynthetic pigments, reduction of ascorbic acid, increased synthesis of phenolic compounds	SO_2, NO_2, Na Cl, heavy metals
Acer platanoides	+, regional and focal necrosis, damage by fungi	+, accumulation of Fe, Mn reduction of photosynthetic pigments, reduction of ascorbic acid, increased synthesis of phenolic compounds	Heavy metals
Quercus sp.	+, necrosis of leaves, reduced vitality, insect damage	+, accumulation of heavy metals	Complex (SO_2, NO_2, heavy metals)
Thuja occidentalis	+, death of needles, dying of shoots, reduced vitality, loss of landings	+, accumulation of heavy metals V, Cr, Fe, Ni, As, and Mo	SO_2, NO_2, Na Cl, heavy metals
Juniperus scopulorum Skyrocket	+, death of needles and shoots, reduced vitality	+, accumulation V, Cr, Fe, Ni, and Sb	SO_2, NO_2, Na Cl, heavy metals
Taxus baccata	+, dying of shoots, reduced vitality, loss of landings	+, accumulation of Cr, Fe, Ni, Zn, As, and Cd in shoots	SO_2, NO_2, Na Cl, heavy metals

The list of species used for biomonitoring and bioindication purposes on a global scale is relatively large. Some of these species are shown in Table 5.1.

5.2 The Use of Higher Plants for Bioremediation (Basic Information)

5.2.1 Phytoremediation

Phytoremediation is the restoration of ecosystems, or their individual components, contaminated with heavy metals, radionuclides, NaCl, petroleum ore, and other toxic organic products by the use of herbaceous and woody plants. The advantage of phytoremediation over other methods of purifying ambient environment is its relatively low cost (50–100 times less), the ability of the implementation in situ, environmental safety, and the ability of further use of obtained biomass to extract valuable elements [108]. One of the drawbacks is the time required for full soil recovery.

Selection of plants for phytoremediation is determined by their ability to absorb toxic compounds from the soil or water systems or transfer them from the surface to volatile forms, the growth rate, and the volume of produced biomass during vegetation and depth of root system.

For phytoremediation, plant hyperaccumulators could be used which accumulate high concentrations of toxic compounds (heavy metals, nonmetals, radionuclides) in biomass [109, 110]. They have developed mechanisms to adapt to high concentrations of metals in organs: representatives of galmain flora (*Viola lutea* var. calaminaria, *Thlaspi* Zn), “tin flora” *Trietaris europea*, *Gnaphalium suaveolens*, accumulating Ni, *Alyssum bertolonii*, *Sebetaria*, copper acuminators *Cyanotis cupricola*, *Sopubia metallorum*, *Gypsophila patrinii*, and others.

Such plants, as a rule, are usually characterized by low biomass. Lately more and more attention of scientists is directed to the use of plants with the medium potential for bioaccumulation of toxic elements, but creating more biomass in the process of vegetation (e.g., C4 plants and woody plants) [111–116]. So far the feasibility of using plants-accumulators of heavy metal is determined by the metal accumulation rate (mg/kg of biomass), multiplied by their biological productivity (kg/ha per year). Economically justifiable plants for phytoremediation are those in which the yield of biomass reaches at least 250 kg/ha per year and metal content in biomass of at least 1% (dry weight) [108].

There are several ways to plant uptake of toxic elements from the environment:

5.2.2 Phytoextraction

Phytoextraction is a process of conversion of heavy metals or metalloids by plants into the form of complex compounds (chelates) and their accumulation in tissues and organs (overground or root system) [117].

To increase the ability of plants to absorb heavy metals from soils, chelators (e.g., ethylenediaminetetraacetic acid, citric acid and oxalic acid, malic acid, salicylate, succinate, tartrate, and other compounds) or inoculation of plants by symbiotrophic microflora (fungi and bacteria), are used [118–120]. Furthermore, the studies are known where the genes of bacterial cells are introduced to the organism of higher plants to increase their ability to absorb heavy metals from the substrate.

The effectiveness of phytoextraction is influenced by several factors: by the content of humus in the soil (the binding of toxic components into complexes increases; their availability for plants decreases), activity of soil microorganisms, introduction of sorbents (iron oxides, manganese, organics, clay, fly ash, coal, vermiculite sawdust, and others) into soil, the pH value of the soil solution (pH reduction leads to an increase in mobility of many heavy metals, Cd, Zn, Ni, etc., as well as binding them with organic soil components), liming (this leads to reduction of the solubility of Fe, Cu, Ni, Co, Zn, and Cd and their availability for plants), introduction of organic acids and complexing agents (enhances uptake of heavy metals by plants), application of plant growth stimulators (heteroauxsin, succinic acid, etc.), and interaction between the ions in the soil solution (formation of insoluble compounds) [106].

5.2.3 Phytotransformation and Phytodegradation

Phytotransformation is an ability of plants to convert toxic compounds (organic pollutants—xenobiotics) in the process of the plant fermentative enzymatic reactions to nontoxic form and their subsequent transfer to the vacuole or binding to lignin and other components of the cell.

5.2.4 Rhizodegradation (Rhizosphere Biodegradation Ore Phytostimulation)

Rhizodegradation (rhizosphere biodegradation ore phytostimulation) is decomposition of toxic organic compounds in the soil in the process of enzymatic degradation in the interaction of the rhizosphere of plants and microorganisms. Thus, the roots of plants affect xenobiotic root exudates, stimulate the increase of the number of microorganisms in the rhizosphere, and accelerate the transfer of toxic compounds in the root zone due to the difference in osmotic pressure between root cells and the soil solution [121, 122]. It is used for soil purification from oil products (not more than 2%): the PAH, PCBs, other hydrophobic aromatic compounds, and pesticides [106].

5.2.5 Phytovolatilization

Phytovolatilization is conversion of toxic components into nontoxic volatile compounds using enzymatic reactions in biochemical cycles of plants and their subsequent release (selenium, mercury) (*Liriodendron tulipifera*) [106].

5.2.6 *Phytostabilization*

Phytostabilization is transfer of metals into the insoluble stable compounds due to synthesis and release by plant compounds that reduce the spread of pollutants (binding to lignin or organic soil components) (conversion into insoluble forms) [123] and precipitation of heavy metals and metalloids (Cd, Cr, Cu, Hg, Pb, Zn, andAs) in the root zone in the form of carbonates, phosphates, and hydroxides. It is used as a step in soil remediation together with the introduction of lime, organic fertilizers, and structurants (phosphates, synthetic resin, clay, bentonite, fly ash, zeolites, aluminosilicates, hydroxides of Fe, Al, and Mn) [106].

5.2.7 *Rhizofiltration*

Rhizofiltration is absorption, concentration, and precipitation of heavy metals and hazardous chemicals by plant roots. This is a most often used technology for water purification from toxic substances and radionuclides. For rhizofiltration rafts in ponds with terrestrial and aquatic plants (in situ) or special tanks for water treatment with platforms (gratings) for plants (ex situ) are used [124–126].

An example of the integrated use of living organisms for bioremediation of water is so called “living machines”: a system of tanks for anaerobic treatment, aerobic treatment using microorganisms and planktonic animals, containers with higher plant hydrophytes and hygrophytes (*Lemna minor, Eichornia crassipes, Phragmites australis, Typha latifolia, Gliceria fluitans, Calla palustris, Alisma plantago-aquatica, Sagittaria* spp., and others), through which the contaminated water flows. As a result, the water is purified from organic and inorganic pollutants.

After rhizoextraction of rhizofiltration, the biomass of plants containing metals can be used for extraction of metals by chemical means (Ni, Cu, Au) or for energy generation [127–129].

At present, scientists intend to create greenbelts of the plant in the industrial zones, which serve as a barrier to heavy metal and serve as phytoremediation of the environment by absorbing heavy metals and radionuclides from the air and soil. The most promising for this are woody plants which possess a combination of features: a deep (or surface) root system of a large volume, a large volume tree crown (height 1.5–30 m), ability to accumulate a large biomass of leaves during the growing season, the possibility of absorption and bioaccumulation of heavy metals (mainly Pb and V) by leaves from atmospheric deposition, durability, and possibility to use wood.

See Table 5.2 for higher plants v.

Many woody plants meet all the requirements of biomonitors listed by Markert:

- High abundance
- Widespread

Table 5.2 Higher plants used for phytoremediation (Baker and Brooks [109]; Wenzel et al. [130]; Glass [131]; Palmer et al. [132]; Prasad [108, 133, 134]; Trace elements [135]; Applied Ecobiotechnology [106]; Favas and Pratas [136])

Species (genera)	Accumulated elements	Substrate
Acacia dealbata	Cu, Pb	Soil
Agrostis tenuis	Cu, Pb, Zn	Soil phytostabilization
Agrostis capillaris	Cu, Pb, Zn	Soil phytostabilization
Agrostis lanatus	Fe, Cu, Zn, As, Pb	Soil
Alyssum sp.	Ni	Soil
Alnus glutinosa	Cu, Pb	Soil
Amaranthus retroflexus, *Amaranthus tricolor*	^{137}Cs, Zn	Soil
Armeria maritima	Pb	Soil
Atemisia absinthium	Zn, Cu, Cr	Soil
Artemisia vulgaris	Zn, Ni, Cu	Soil
Atriplex prostrata	NaCl	Soil
Alisma plantago-aquatica, *Calla palustris*, *Gliceria fluitans*, *Sagittaria* spp	HM, organic compounds	Storm water ditch, sewage wetlands, water
Berberis	Xenobiotics	Soil rhizodegradation
Beta vulgaris	Ni, Cu, Zn, Cr	Soil
Brassica canola	^{137}Cs	Soil
Brassica juncea	Pb, Zn, Cr, Cd, Ni, Cu,^{90}Sr, Se, U	Soil phytoextraction (U—only with organic acids), phytotransformation (Cr^{+6}-Cr^{+3}); phytostabilization
Brassica nigra	Zn, Pb	Soil
Buxaceae	Ni	Soil
Calamagrostis epigejos	Pb, Zn, Cu	Soil
Cardamonopsis hallerii	Heavy metals (HM) (Zn, Cd)	Soil, hydroponics
Cynodon dactylon	Xenobiotics	Soil rhizodegradation
Eucalyptus sp., *Eucalyptus globulus*	Na, As, Cu, Pb	Soil
Eichhornia crassipes	Pb, Cu, Cd, Fe	Storm water ditch, sewage wetlands, water
Festuca arundinacea	Xenobiotics	Soil rhizodegradation
Festuca rubra	Pb, Zn	Soil phytostabilisation (with $CaCO_3$)
Fagopyrum esculentum	Ni	Soil
Juncus compressus	Zn, Cd, Pb	In the roots
Haumaniastrum katangense	Co	Soil
Chenopodium album	Zn, Cu	Soil
Helianthus annuus	Cr, Mn, Cd, Ni, Zn, Cu	Soil, storm water ditch, sewage wetlands, water (rhizofiltration)
Helianthus annuus	Pb, U, ^{137}Cs, ^{90}Sr, Cu (mutant forms)	Soil
Hydrocotyle umbellata	Pb, Cu, Cd, Fe	Soil
Kochia scoparia	Radionuclides (RN)	Soil

(continued)

Table 5.2 (continued)

Species (genera)	Accumulated elements	Substrate
Lemna minor	Pb, Cu, Cd, Zn	Storm water ditch, water
Linum usitatissimum	Cu, Ni, Cd, Cr, Pb	Soil (phytoremediation + raw material for plant fiber)
Lycopersicon lycopersicum	Pb, Zn, Cu	Soil
Lolium perenne	Radionuclides, xenobiotics	Soil rhizodegradation
Medicago sativa	Ni (HM), Pu, xenobiotics	Soil (symbiosis with bacteria)
Melilotus officinalis	Zn, Ni, Cu	Soil
Miscanthus giganteus	Cu, Ni, Cd, Cr, Pb	Soil (phytoremediation + raw material for plant fiber)
Morus sp.	Xenobiotics, HM	Soil rhizodegradation
Phalaris arundinacea	Cd, Cu, Zn, Pb	Storm water ditch, sewage wetlands, water
Polygonum sp., *P. sachalinense*	Cd, Pb, Zn, ^{137}Cs, ^{90}Sr, Cu	Soil
Populus sp.	Hg, Fe, Ni, Zn, Cd, Pb, herbicides	Soil phytoextraction, soil rhizodegradation
Pinus pinaster	Fe, Zn, As, Pb, W	Soil
Phalaris arundinacea	Cd, Cu, Zn, Pb	Storm water ditch, sewage wetlands, water
Quercus ilex, *Quercus rotundifolia*, *Quercus suber*	Ni, As, W, Zn, Pb	Soil
Rhus typhina	Polycyclic aromatic hydrocarbons	Soil rhizodegradation
Salix sp.	Ni, Zn, Cd, Pb, perchlorate	Waste water, filtrates
Scirpus sylvaticus	Cd, Cu, Zn, Pb	Storm water ditch, sewage wetlands, water
Secale cereale	Zn, Pb	Soil (only with the introduction of bacteria *Rhodococcus equi*)
Silene latifolia	Zn, Cu	Soil
Sorgo bicolor	Zn, Cu, Pb	Soil
Trifolium sp.	Xenobiotics	Soil rhizodegradation
Typha latifolia	Cu, Zn, Cd, Pb	Storm water ditch, sewage wetlands, water
Thlaspi caerulescens	Zn, Cd	Soil
Thuja occidentalis	V, Cr, Fe, Ni, As, Mo	Soil
Urtica dioica	Cu, Ni, Cd, Cr, Pb	Soil
Vetiveria zizanioides	Cr, Cu, Ni, Zn, As, Cd, Pb	Water, soil

- Easy to identify
- Easily available
- Analytically accessible and low detection and determination thresholds with current analytic technology
- Accumulation of pollutants

And features mentioned by Bargagli [3]:

- Long vegetation period
- Clearly marked and reproducible response to certain changes in the environment

In addition, they have a number of advantages for phytoremediation [136, 137]:

- A high yield of biomass (at a density of 10,000–20,000 per hectare) to 15 tonnes of dry matter/ha per year, which enables efficient phytoextraction with moderate amounts of accumulation of toxic elements [138]
- Famous cultivating agricultural technology (*Salix* and *Populus* trees grown on a short rotation system: the harvest in 3–5 years with a total duration of 30 years of cultivation), which can be adapted for use at contaminated lands
- Ability after bioremediation to be used as biofuels (direct combustion, anaerobic digestion processing, fermentation in liquid fuels), for the production of wood, ethanol, biogas, biofortified, biochar, chipboard, paper, constructions, and technical production [139–141]
- Ability to use in urban landscapes
- Creation of greenbelts and phytocenoses for remediation [142–144]
- The stabilization of the substrate under cultivation: soil protection from water and air erosion; prevent metal leaching to protect surface and groundwater

5.3 Possibilities of Woody Plant Use for Biomonitoring of Antropogenic Pollution of Environment

Selection of species for environmental assessment is dictated by a number of necessary conditions: they must be sufficiently widespread in the study area and well reflect the state of the environment by changes of qualitative or quantitative characteristics (e.g., the development of necrosis and chlorosis, the change of physiological parameters, morphological or anatomical changes, changes at the molecular level, etc.). Based on these characteristics the assessment of the environment can be carried out which includes physiological and biogeochemical characteristics of species [19–23, 34, 35, 88–105].

An important issue is the expansion of the list of species of woody plants, which can be used for phytoremediation of environment in conditions of complex pollution of air and soil by heavy metals in industrial centers.

We carried out integrated monitoring of ecosystems with varying degree of anthropogenic load at the territory of a model region of the central zone of Russia—Tula region. The parameters of the woody plants growing in natural habitats (forests and forest-steppe ecosystems) and in polluted urban environment of the regional center of industry (protection zones of motorways, the territory of the metallurgical enterprises) were determined [103].

The parameters of bioaccumulation of toxic elements of trees growing in contaminated and clean areas of Tula region (Russia) were studied.

5.3.1 Study Area: Objects of Investigation

The model region of the study was Tula region and the city of Tula. In the study area, three natural territories are located: coniferous-deciduous forests in the north, deciduous forests in the center of the region, and forest steppe and steppe in the south. The region is characterized by a well-developed industry: mechanical engineering and metal working, chemical industry, defense industry, ferrous metallurgy, construction materials, light industry, and food industry. Districts of Tula region are characterized by a varying degree of anthropogenic impact. Tula industry (ferrous metallurgy and mechanical engineering) and Novomoskovsk (chemistry) account for more than 2/3 of the regional production. A great contribution to the pollution of the region is from the chemical industry centers Schekino, Efremov, and Aleksin and the center of the electricity Suvorov. The remaining 20 districts of the region account for 10% of industrial production. According to the concentration of industrial enterprises, the Tula region is the second after Moscow, and it is among the five most ecologically unfavorable regions of Russia, ten times exceeding the amount of emissions to the atmosphere of the surrounding Kaluga and Oryol regions. 94% of all emissions are due to the city of Tula and Aleksinskiy, Suvorovskiy, Efremovskiy, Novomoskovskiy, Uzlovsky, and Schekinsky districts where the largest number of industrial enterprises is clustered. 52% of pollutants in the atmosphere fall to the share of industrial enterprises.

The regional center—the city of Tula—is located 180 km south of Moscow. This ecosystem includes the city area of 154 km^2 and a population more than 500,000; it represents an area with developed metallurgical, chemical, engineering, and defense industries with the city's infrastructure and network of roads with heavy traffic. The first stage of investigations was focused on revealing the geochemical anomalies of soil and the analysis of atmospheric air. The results of these investigations showed that more than 40% of the territory of the selected ecosystem was characterized by excess of maximum permissible levels (MPL) of the set of heavy metals in the environment (Fig. 5.1).

The main element pollutants of urban (Tula city) soils were:

Mn (in sampling point 1 up to 50% of soil exceeded MPL)
Fe (high gross concentration all over)
Cu (24% soil exceeded MPL up to 3–6 times)
Zn (28% of soil showed excess of MPL by 15–62%)
As (38% of soil excess of MPL by 36–62%)
Pb (12% soil excess of MPL by 10–50%)

The total index for grading soil contamination identified 20 areas of moderately hazardous category (28% of soils) and 4 of extremely dangerous category (6% of soil) [145]. The map (Fig. 5.1) presents geochemical anomalies in soils of the city on the total pollution index. In the most polluted areas, the analysis of atmospheric air was carried out. The high content of Fe in the form of oxides and sulfates at all sampling points was revealed, which exceeded the MPL average concentrations of Fe by several hundreds of times. The copper concentration was higher than the

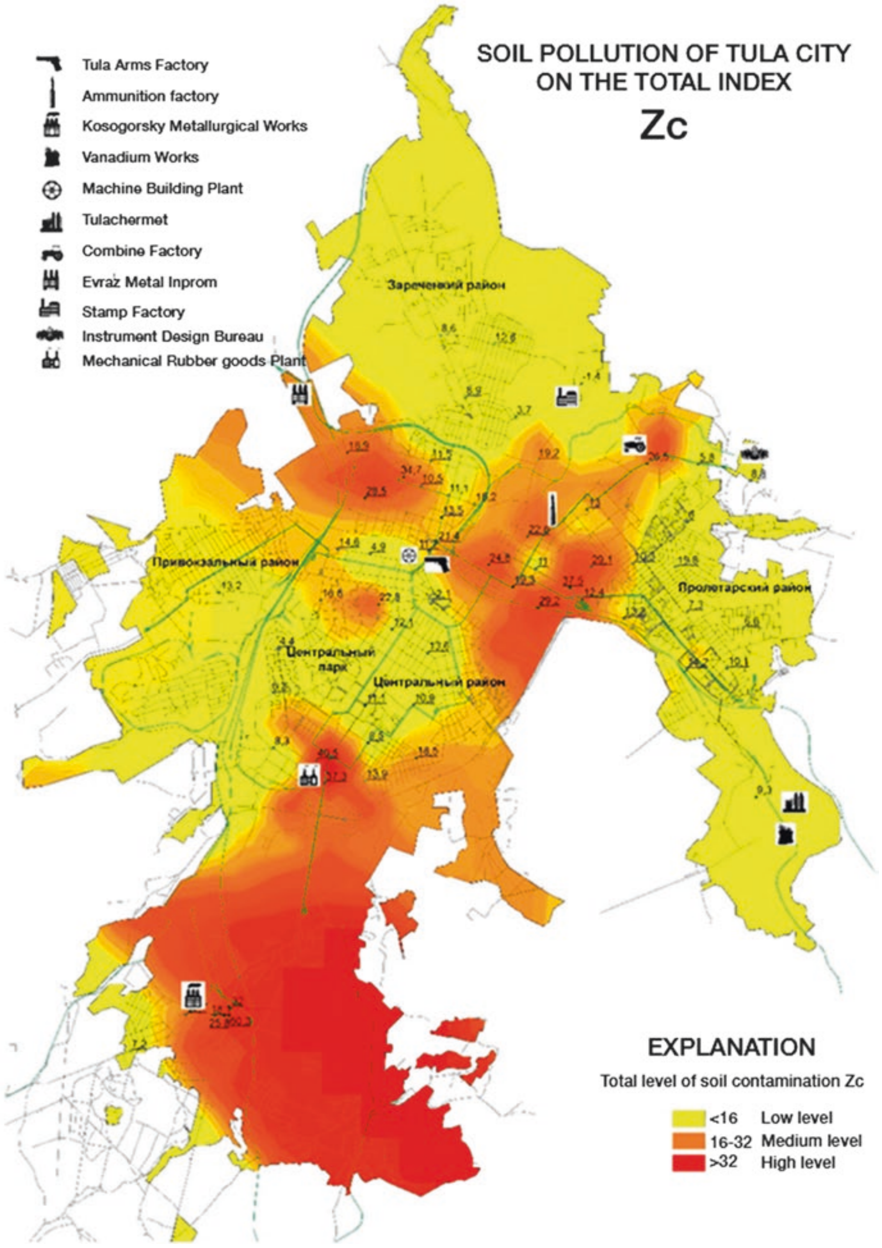

Fig. 5.1 Characterization of Tula city soil pollution

maximum single MPL by 56% of the surveyed zones and exceeded the daily average by 1.5–3.3 times and maximum single—in 3–9 times. Pb content exceeds the average daily rate of MPL sampling points close to Kosogorsky Metallurgical

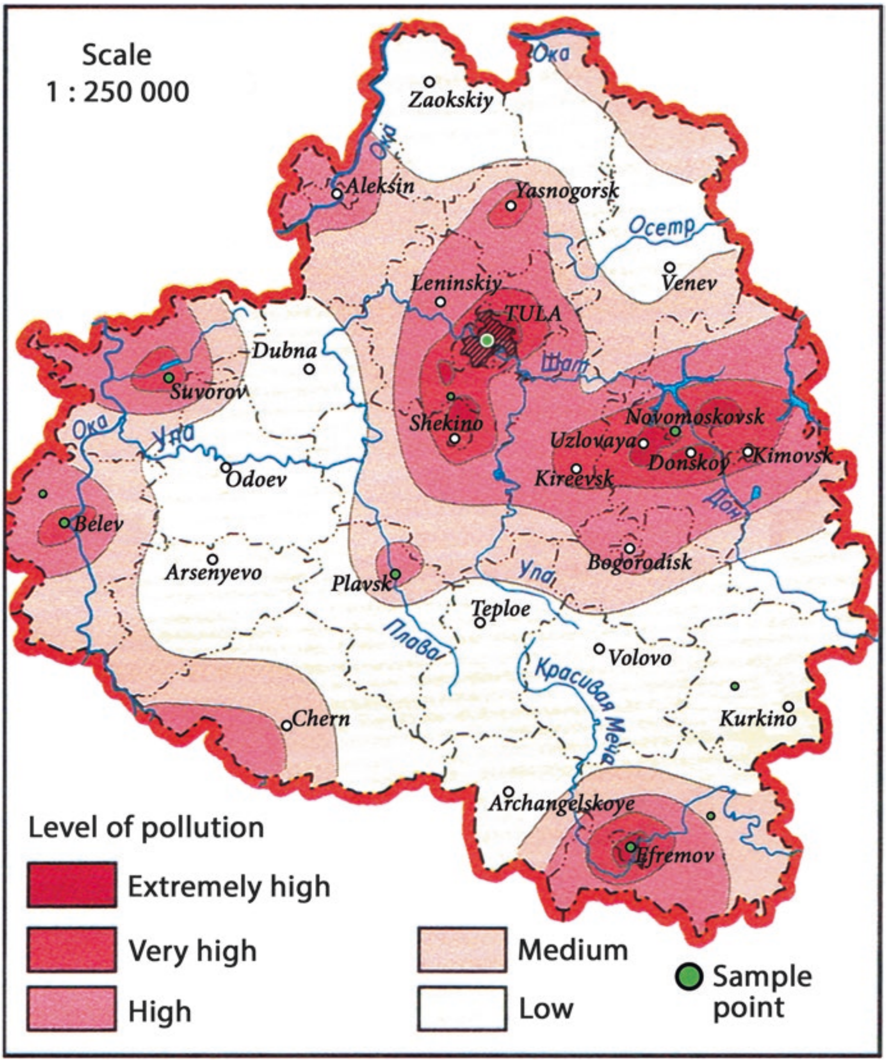

Fig. 5.2 Characterization of Tula region soil pollution and sampling points

Works (ferromanganese production), the Mogilev square (Pedagogical University), and at the intersection of two main roads of the city (Krasnoarmeyskiy Avenue) [104, 105, 145].

To investigate the possibility of using woody plants for biomonitoring the environmental situation, seven areas with different levels of anthropogenic pollution of the region and the regional center—the city of Tula—were chosen (Fig. 5.2, Table 5.3). The objects of biogeochemical parameters in the study (content of elements in the leaves) were native tree species: *Tilia cordata*, *Acer platanoides*, *Salix fragilis*, and *Picea abies.*

Table 5.3 Characterization of different areas of Tula region

Sampling point	Description of environmental conditions
City of Tula, sanitary-protective plantings along roads	Industrially developed urban ecosystem with a high level of technogenic pollution, exceedance of MPL of HM metals in soils by 40% of the territory, a high level of dust and exceedance of the MPL of HM in the air sampling points
Tula, sanitary-protective zone of metallurgical enterprises, KME and Tulachermet	In soil samples of the sanitary-protective plantings observed, an excess of MPL of HM on a number of elements, Mn (twofold), Pb (1.5 times), and Zn (2 times), was observed
Kulikovo Pole	The area is located in forest-steppe vegetation zone of Tula region and characterized by low level of human impact (a historical place reserve museum area). Agricultural using with fertilizers is the main form of anthropogenic activity for the soils
Yasnaya Polyana	The area of museum reserve "Yasnaya Polyana" is located in deciduous forests and influenced of metallurgical and chemical enterprises (Kosogorsky metallurgical plant, Shchekinoazot)
Plavsk town	The city is located in the forest-steppe part of the region. The state of ecology is affected by a distillery "Plavsky" emissions of which contaminate the river
Belev town	The city is located in coniferous-deciduous forest area and experiences recreational and vehicle load, and among the industrial enterprises is the plant Transmash
Belevskiy area (forest)	The region is located in a strip of coniferous-deciduous forests, and there are no large industrial enterprises
Novomoskovsk town	The city with high level of industrial pollution (Nitrogen, Procter & Gamble—Novomoskovsk, Knauf Gypsum Novomoskovsk, Orgsintez, Polyplast, Novomoskovskaya GRES)
Suvorov town	The industrial city is located in coniferous-deciduous forest area and influenced of Cherepetskaya hydropower station, Cherepetskaya precast concrete plant, Mitinskaya Iron Works and recreation
Suvorovskiy area (Varushizi)	The city is located in coniferous-deciduous forest area and influenced of Cherepetskaya hydropower station
Efremovskiy area (Shilovo)	The region is located in the forest-steppe part of the region. The enterprises have a negative impact on the environment and are the production of synthetic rubber and household chemicals (Novomoskovskbytkhim and Procter & Gamble) and Efremov thermal power station

The sampling sites to determine the suitability of species for phytoremediation of soils in sanitary-protective planting of metallurgical enterprises of Tula are: point I, JSC "Kosogorsky Metallurgical Works" (KME) (ferromanganese production), and point II, complex of enterprises of JSC JV "Tulachermet" and "Vanadium" (Tulachermet) (production of pig iron, vanadium, and chromium). For relatively pristine (background or control) zone, the area of the Central Park of Culture and Leisure was chosen. The distance between point I and control zone is 2–3 km and between point II and control zone is 5–6 km. Distance of sanitary-protective planting from aerosol emission sources is 30–400 m.

The objects of investigation of woody plant feasibility for bioremediation are seven tree species and eight shrubs dominating in the sanitary-protective zone of the metallurgical enterprises: *Sorbus aucuparia*, *Acer platanoides*, *Populus nigra*, *Aesculus hippocastanum*, *Tilia cordata*, *Larix sibirica*, *Betula pendula*, *Crataegus sanguinea*, *Crataegus monogyna*, *Cornus alba*, *Cotoneaster lucidus*, *Symphoricarpos albus*, *Syringa vulgaris*, *Philadelphus coronarius*, and *Physocarpus opulifolius.*

5.3.2 Sampling, Sample Preparation, and Methods of Research

Sampling to determine the ability of wood to bioaccumulation of toxic elements was carried out in the third decade of July during the vegetation peak over the perimeter of the tree crowns at a height of 1.5–2 m in the plant communities of different districts of the region and urban ecosystems of the city of Tula. The minimum number of trees (shrubs) in each type of sampling points was ten. The minimum number of leaves from each tree (shrubs) was ten.

Leaves of woody plants were washed in running water, and then they were washed twice in distilled water. This way of sample preparation, as opposed to the use of unwashed samples, in our opinion, allows to avoid large errors in sample preparation that may occur due to loss of the dust particles in the course of operations, packaging, grinding, weighing, and pressing samples and eliminates dependence on climatic factors (washings by rains, the wind emission) during the sampling and before it. It allows to perform a comparative description of the research results.

Washed samples were dried at room temperature and brought to constant weight in an oven at a temperature of 60 °C. The samples were averaged and were packed in paper bags with a label. Sample preparation for instrumental neutron activation analysis (INAA) (grinding, weighing, pressing, and packing containers of samples) took place in a chemical laboratory sector neutron activation analysis LNP JINR.

Part of the elements in plant samples (Mn, Fe, Ni, Cu, Zn, Cd, Pb) was determined in the laboratory of chemical analysis of the Geological Institute (GIN RAS) by atomic absorption spectrometry using "QUANT-2A" (KORTEK, Moscow) equipped with deuterium corrector of nonselective absorption and relevant hollow-cathode lamps; determination of heavy metals in the samples was carried out in accordance with standardized methods [146]. Determination of Zn, Pb, Cu, and Cd was performed in "propane-air" flame and Fe, Mn, and Ni in "acetylene-air" flame. Quality control was provided by using certified reference materials IAEA-SOIL-7, IAEA-336 (lichen), SRM 1572 (*citrus* leaves), and SRM 1575 (*pine* needles).

INAA of plant samples was carried at the IBR-2 reactor at JINR LNP using activation with epithermal neutrons along with the full energy spectrum. To determine the long-lived isotopes, samples of leaves of about 0.3 g were packed in aluminum foil. The containers with samples were irradiated for 4–5 days in a cadmium-screened channel (epithermal neutron activation analysis). After exposure, the samples were repacked in clean plastic containers for measurement of induced activity.

Induced gamma activity of the samples was measured twice: after 4–5 days after irradiation (for determination of As, Br, K, La, Na, Mo, Sm, U, and W) and after 20 days (to determine Ba, Ce, Co, Cr, Cs, Fe, Hf, Ni, Rb, Sb, Sc, Sr, Ta, Tb, Th, Yb, and Zn). Measuring time was 40–50 min and 2.5–3 h, respectively.

To determine the short-lived isotopes of elements (Al, Ca, Cl, I, Mg, Mn, and V), samples of 0.3 g weight were packed in polyethylene packs and irradiated for 3–5 min. Induced gamma activity of the samples was measured after 5–7 min of cooling twice: for 3–5 and 10–5 min, successively.

Measurement of the induced gamma activity was carried out by gamma spectrometers with Ge (Li)—detectors with a resolution of 2.5–3 keV of the gamma line 1332 keV of ^{60}Co and HPGe detector with a resolution of 1.9 keV of the gamma line 1332 keV of ^{60}Co.

A software package developed in the Frank Laboratory of Neutron Physics of JINR was used for processing gamma spectra of induced activity and calculating the elemental concentrations. The concentrations of elements were determined by relative methods (by comparison with the standards) [147]. Certified reference materials (pine needles, NIST) were irradiated and measured together with samples.

The uncertainties in elemental determinations of Na, K, Cl, As, Sr, Fe, and Pb are in the range of 5–10% and for V, Ni, Cu, Se, Mo, Cd, and Sb are 30%.

So far for vegetation there are no identified MPLs and the data on the elemental content in the different studies are very different in dependence on the used methods and sample preparation [21, 34, 90–95] (some studies are made using unwashed plant material), to assess the biochemical characteristics of the investigated samples, they were compared with the average data of the Reference plant (RP) [148].

5.3.3 Results and Discussion

The results carried out in seven districts of the Tula region showed that the leaves of woody native species can be used as bioindicators and biomonitors of biogeochemical parameters in determination of the degree of anthropogenic load on ecosystems.

The two studied species in the cities accumulate more chlorine in the leaves than in the steppe and forest communities. Thus, the chlorine content in the leaves of *Tilia cordata* and *Acer platanoides* in the towns of Plavsk, Novomoskovsk, and Tula varied in interval of 3270–6400 mg/kg, that is, 1.5–3 times higher than the critical concentrations and mean values for vegetation [148–150] and 2–17 times higher than the values for forest and steppe of the region (370–1520 mg/kg) (Fig. 5.3). The accumulation of high concentrations of chlorine in leaves of trees in urban areas may be due to the use of NaCl on the sidewalks followed by washing the salt melt water into the soil in winter and early spring as well as by deposition of the aerosol particles due to the impact of the chemical industry.

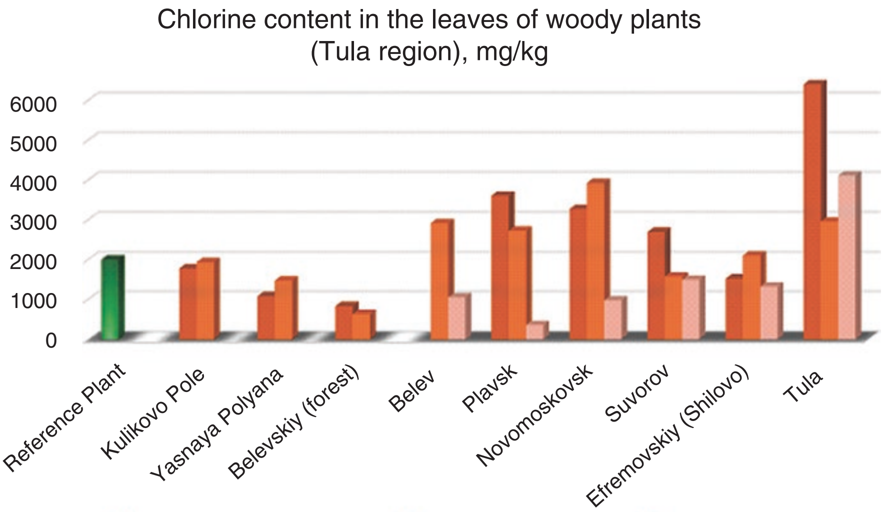

Fig. 5.3 The chlorine concentration in the leaves of woody plants from Tula region ecosystems with varying degrees of anthropogenic load

Acer platanoides, *Salix fragilis*, and *Picea abies* well reflect an increase in the concentration of vanadium in the environment. So, in Belev and in deciduous forest near the city, as well as in the city of Tula (sanitarian-protective zone of motorways), V content in the leaves and needles of the enlisted species ranged from 0.7 to 1.5 mg/kg of dry matter, that is, 1.3–3 times higher than in the reference plant (RP) (0.5 mg/kg). It could be connected with aerosol emissions from enterprises of "Vanadium," Instrument Design Bureau, NGO "Fusion" (Tula), and JSC "Transmash" (Belev) (Figs. 5.4, 5.5, and 5.6).

The concentration of chromium in leaves of all investigated deciduous woody plants was 1.5–2.7 times higher than in RP (2.3–5.0 mg/kg dry weight) at all sampling points examined except Belevskiy area (Figs. 5.4, 5.5, and 5.7). This fact may be an evidence of air emission of this element, and it also confirms our previous assumption that woody plants concentrate more chromium in organs rather than herbaceous plants [38–40, 148, 149].

The given fact evidences that the woody plants are sensitive indicators to the content of the given element in the environment in time and they can reflect the spatial distribution of an air emission and absorb elements from deeper soil horizons.

Two deciduous species *Acer platanoides* and *Salix fragilis* react to high iron content in the soil and air (Figs. 5.4 and 5.5). The greatest sensitivity characterizes *Acer platanoides* (element content of the leaves increases up to 1250 mg/kg (Tula), that is, twofold higher than the concentrations of toxic element for vegetation [149, 150] and eight times higher than the RP). Such intense absorption of iron along with other heavy metals can lead to the development of necrotic changes in the leaf and to reduction of vitality of the species in terms of polymetallic soil pollution of industrial cities.

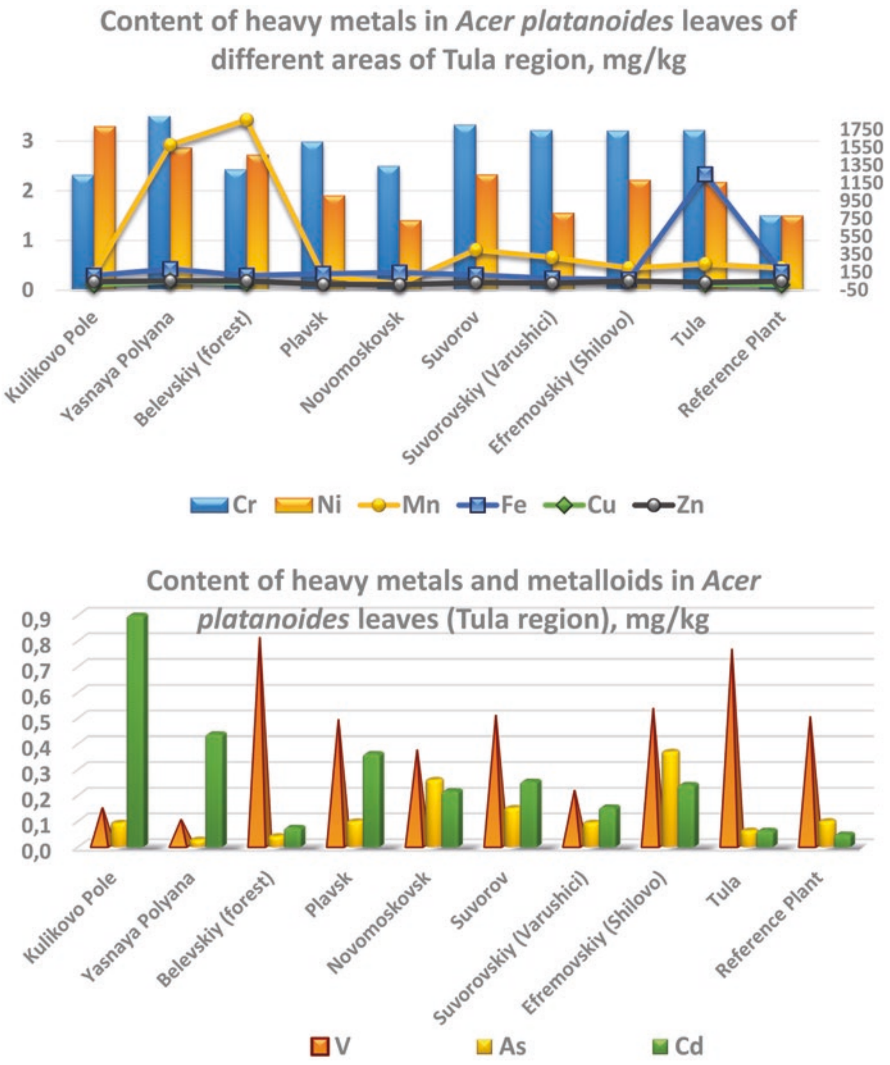

Fig. 5.4 The content of heavy metals and metalloids in *Acer platanoides* leaves grown in different areas of Tula region

Bioaccumulation of copper in leaves of woody plants in Belevskiy area (city and forest), Yasnaya Polyana, and the regional center—the city of Tula—reached 24–53 mg/kg of dry weight, that is, 2.5–5 times higher than the values of RP. The high concentration of the element in the wood plants is conditioned by its high content in the air and soil due to the impact of metallurgical industry and metalworking [104, 105, 145].

Accumulation of arsenic was noticed in the leaves of deciduous trees at the sampling points in Novomoskovsk and Efremovskiy area (Shilovo village) (Figs. 5.4 and 5.7). Its concentrations of 0.21–0.37 mg/kg of dry weight are 2–3.5 times higher than in the reference plant. *Picea abies* needles accumulate in two times less arsenic than RP regardless of the point of sampling (Fig. 5.6).

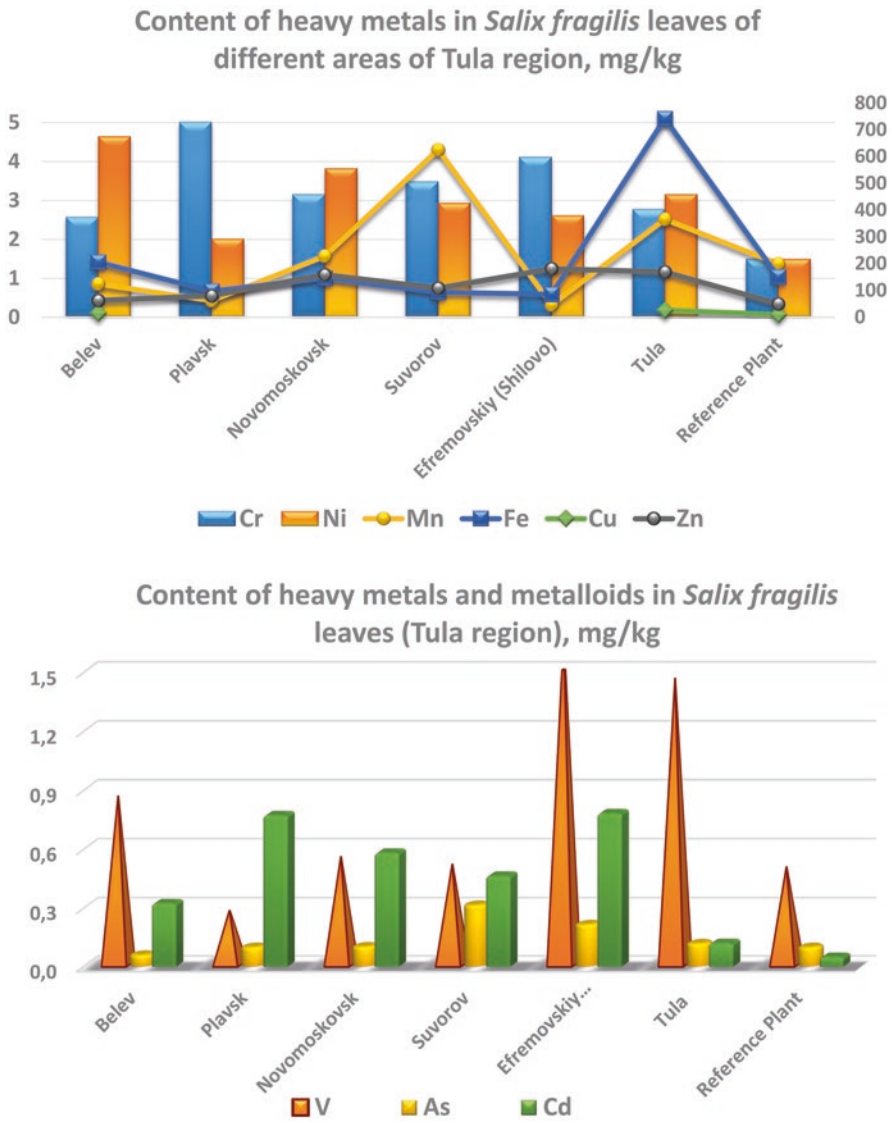

Fig. 5.5 The content of heavy metals and metalloids in the leaves of *Salix fragilis* (Tula region)

The trend in accumulation of cadmium is similar to the arsenic one: *Picea abies* does not accumulate element in the needles, while all deciduous woods are investigated at all points, except with sampling growing in sanitary-protective zone along Tula roads and in the forest area of Belevskiy, accumulated from 0.16 to 0.92 mg/kg of the element in the needles that exceeded by 3–18 times the average data for plants (Fig. 5.6). The highest concentration of the element was observed in *Acer platanoides* leaves growing in the Kulikovo Pole, as well as in the leaves of *Salix fragiles* (Figs. 5.4 and 5.5). The best biomarker for this element is *Salix fragilis*. The fact of high

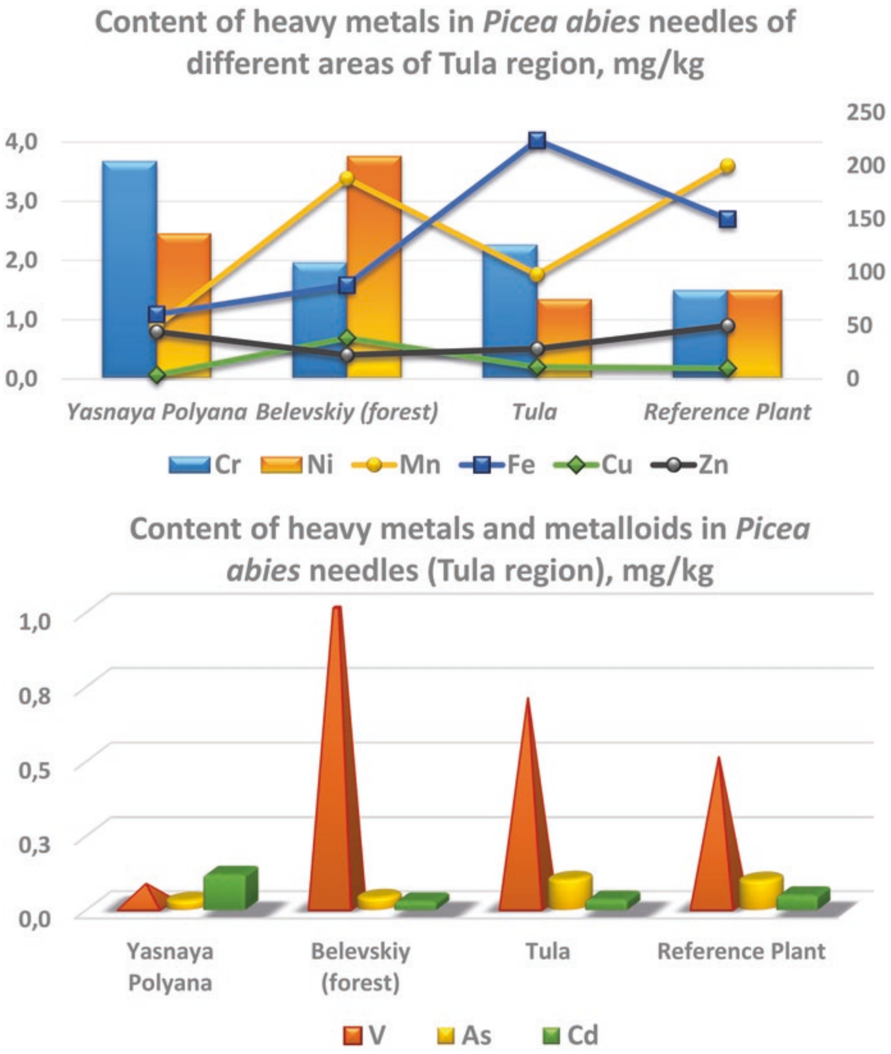

Fig. 5.6 The content of heavy metals and metalloids in the needles of *Picea abies* in different ecosystems of Tula region

accumulation of cadmium by leaves of woody plants can be explained by air emission of the element by motorway, as well as, apparently, by better degree of absorption of the elements at alkaline soils (Efremovskiy area (Shilovo), Kulikovo Pole). The trend of low bioaccumulation of cadmium in leaves of trees growing in Tula, in the soils of multi-element anomaly, can be explained by the antagonism of the ions when accumulated from the environment, as well as the low level of cadmium in the soil.

Taking into consideration the difference in bioaccumulation of toxic elements by leaves of plants growing in different districts of the region, one may conclude that the chosen plant for bioindication and biomonitoring reflects the environmental

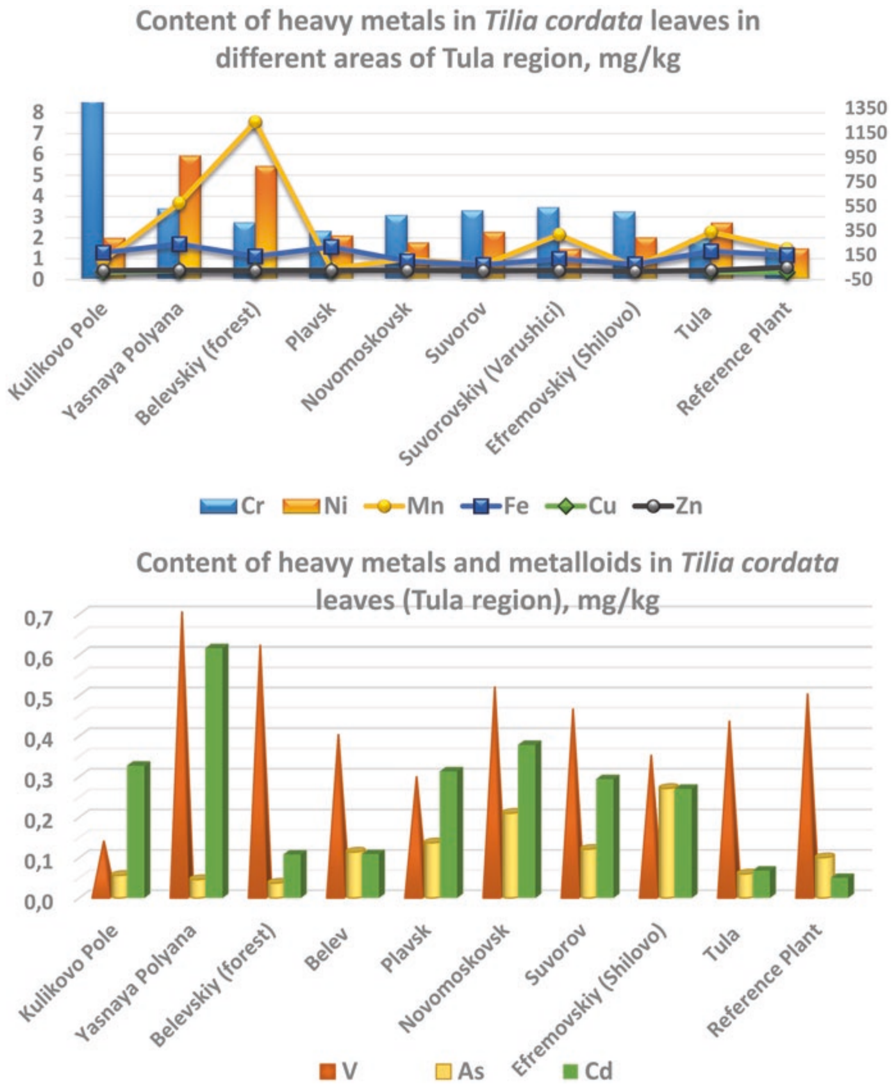

Fig. 5.7 Heavy metal bioaccumulation in leaves of *Tilia cordata* (Tula region)

situation in ecosystems with different levels of anthropogenic load and can be used as biomonitors.

At the same time the largest range of changes in biogeochemical activity is typical for species *Acer platanoides* and *Salix fragilis*, which are preferably used for biomonitoring based on biogeochemical parameters of variability options when changing of the main sources of environmental pollution occurs. Aboriginal species of Gymnospermae—*Picea abies*—is less favorable for the chosen purposes. Below are details of the biogeochemical activity of the studied species (Table 5.4).

Table 5.4 Biogeochemical activity of leaves of woody plants reflecting the anthropogenic load on ecosystems

Species	Elements which accumulated at elevated technogenic loads
Acer platanoides	Cl, V, Mn, Fe, Ni, Cu, As, Cd
Tilia cordata	Cl, Mn, Ni, Cd
Salix fragilis	Cl, V, Mn, Fe, Ni, Zn, As, Cd
Picea abies	V, Mn, Fe

Analysis of correlation bindings at bioaccumulation of elements by leaves of plants revealed strong correlation between accumulated elements. Al correlates with V, Zn, Sm, Hf, Th, and U (accumulation of elements of technogenic soil contamination); Mn with Cu; Cd with W; V with Th and U; and Zn with Th and U (impact factor of metallurgical defense and metal processing industries).

5.4 Accumulation of Heavy Metal in Conditions of Polymetallic Contamination of Industrial Areas (Metallurgical Plants) by Woody Plants of Moderate Climate: Possibilities of Their Application for Soil Phytoremediation

At present a lot of investigations on the use of woody plants for phytoremediation of soil from heavy metals were undertaken. The genera *Salicaceae: Populus* and *Salix* [113, 137, 151–158], *Pinus* [136, 159–161], *Acer* [162, 163], *Betula* [164–168], *Quercus* [136, 169, 170], *Morus alba* [115], *Acacia retinoides* and *Eucaliptus torquata* [171, 172] for soil of subtropical climate were investigated.

For phytoremediation of the environment from radionuclides the resistant to them *Juglans mandshurica* and characterized by high ecological plasticity *Phellodendron amurense* were used.

An important issue is the expansion of the list of species of woody plants, which can be used for phytoremediation of environment in conditions of complex air and soil heavy metal pollution in industrial centers (in a temperate continental climate).

Prior to the beginning of our biogeochemical studies, we have evaluated the vitality of species of sanitary-protective plantations of metallurgical enterprises and highways, the presence of necrotic and chlorotic leaf damage, as well as their ability to accumulate dust emissions on the leaf surface.

In assessing the vitality, the scale was proposed by T. V. Chernenkova [94]: 1 point, “healthy”; 2 points, “weakened”; 3, “severely weakened”; 4 points, “moribund”; and 5 points, “deadwood.” Assessment of the vitality showed that the most morphologically adapted species to the conditions of polymetallic contamination are *Larix sibirica*, *Syringa vulgaris*, *Caragana aerorescens*, *Ligustrum vulgare* (vitality 1), *Philadelphus coronarius* (vitality of 1–2), *Sorbus aucuparia*, and *Acer platanoides* (vitality 2). All studied species in the sanitary-protective plantations showed necrotic and chlorotic leaf change (from 7 to 98%—the most in the genus *Populus*), expressed in the point and edge necrosis and interveinal chlorosis.

Fig. 5.8 Necrosis and chlorosis of tree leaves growing in the area of influence of metallurgical enterprises (*Sorbus aucuparia*, *Aesculus hippocastanum*, *Tilia cordata*)

Manifestation of damage in the leaves of various species was different. For example, *Sorbus aucuparia*, *Betula alba*, *Acer platanoides*, *Tilia cordata*, and *Cotoneaster lucidus* showed the appearance of the point of necrosis on the leaf blade (Figs. 5.8, 5.9, and 5.10). In *Aesculus hippocastanum, Cotoneaster lucidus, Crataegus, Cornus alba*—edge and interveinal necrosis of leaves appear (Figs. 5.8 and 5.10).

All these symptoms may be indicative of the direct damage of leaf tissue by toxic concentrations of iron, manganese, nickel, and chromium [34]. The development of necrosis of leaves can be used for bioindication of the environment at affected areas by the polymetallic pollution with the help of the leaves of wood. Leaf chlorosis may be caused by an imbalance in the leaves with an excess of magnesium ions of other substituents. At the level of the leaf anatomy noted manifestation of was observed kseromorphism symptoms (reduction of the leaf square, Fig. 5.9), An increase in the number of stomata, sheet thickness and diameter of the stomata to compensate for exchange in dusty conditions and high concentrations of heavy metals in the environment of the sampling sites took place [35].

The ability to accumulate dust by leaves of woody plants is different for trees and shrubs and is ranging from 8 to 206 mg/dm^2 for trees and from 17 to 423 mg/dm^2 for shrubs.

The maximum ability to accumulate dust in sanitary-protective plantations belongs to:

- *Tilia cordata* (till 115 mg/dm^2)
- *Populus nigra* (till 115 mg/dm^2)
- *Salix caprea* (till 141 mg/dm^2)

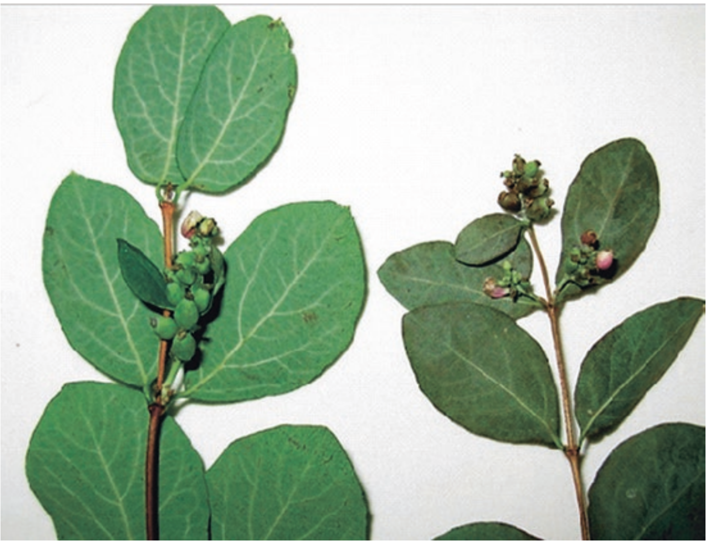

Fig. 5.9 Decrease of leaf area of the *Symphoricarpos albus*, which grows in the area of influence of metallurgical enterprises (*right*) in comparison with the control zone (*left*)

Fig. 5.10 Necrosis and chlorosis of shrub leaves growing in the area of influence of metallurgical enterprises (*Cornus alba*, *Cotoneaster lucidus*, *Crataegus monogyna*)

- *Sorbus aucuparia* (till 206 mg/dm^2)
- *Crataegus sanguninea* (till 107 mg/dm^2)
- *Philadelphus coronarius* (till 149 mg/dm^2*)*
- *Symphoricarpos albus* (till 153 mg/dm^2)
- *Syringa vulgaris* (till 189 mg/dm^2*)*
- *Cornus alba* (till 296 mg/dm^2)
- *Ligustrum vulgare* (till 423 mg/dm^2*)*

We have determined the dust particle content on the surface of leaves using the method of electron scanning microscopy in the laboratory of geochemistry and mineralogy of soil of Federal State Institute of physical, chemical, and biological problems of pedology of RAS (operator E. I. Elfimov).

The distribution of elements on the leaves surface is random, but the greatest number of dust particles concentrates at the bottom, along the edge of the leaf surface and along the main veins of leaves (Fig. 5.11). Analysis of the spectra showed

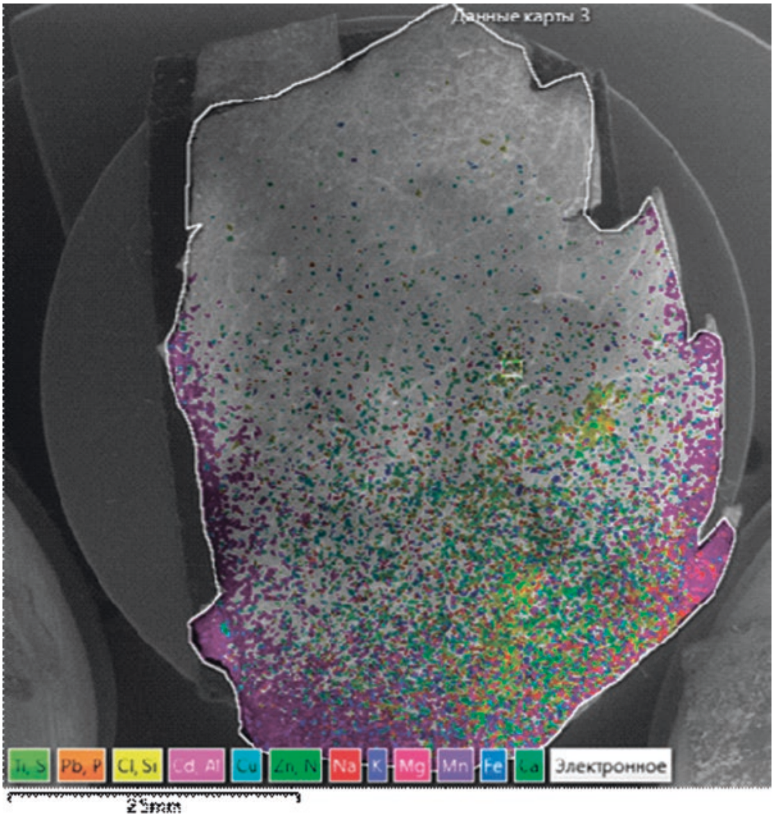

Fig. 5.11 Distribution of dust emissions on a fragment of leaf surface of *Crataegus sanguinea* which grows in the area of metallurgical production (scanning electron microscope)

that most of the adsorbed particles are those compounds of organic nature, and about 1–3% accounted for by soil components, the proportion of heavy metals Fe, Cu, Mn, Cd, Zn, and Pb on the leaf surface may vary from 0.02 to 5% for each element. The method might be the qualitative for analysis of components of aerosol emissions and allows one to establish the presence and ratio of the components on the surface of the leaf that reflects the air pollution of the studied zone.

5.4.1 Bioaccumulation of Heavy Metals by the Trees and Shrub Leaves

5.4.1.1 Manganese

Analysis of the Mn content in leaves of woody plants showed that its concentration ranges from 23 to 385 mg/kg (Table 5.5, Fig. 5.12). That is a rather strong variation of the element probably depending on the species features, growing conditions, and the element concentrations in the environment . Low concentrations of the element (23–150 mg/kg) are characteristic for species such as *Aesculus hippocastanum*, *Tilia cordata*, *Betula pendula*, *Cornus alba*, *Physocarpus opulifolius*, and *Philadelphus coronarius* growing in urban conditions in relatively pristine areas. However, the impact of emissions from the steel industry resulted in increase of the content of elements by two times in *Philadelphus coronarius* and *Tilia cordata* (sample point KME), four times in *Aesculus hippocastanum*, and eight times in *Betula pendula* and reached a value of 340 mg/kg, that is, on average, 1.5 times higher than in the reference plant [148]. However, for *Larix sibirica* in excess of the concentration of the element in the environment overrelatively permissible concentrations, increase of its content in the leaves was not noticed. This may serve as evidence for inclusion of protective mechanisms in the absorption and transport of this element by plant root system, and it also can be caused by antagonism with Fe ions in the process of element uptake by root system.

The role of *Betula pendula*, *Pinus sylvestris*, and *Larix sukaczewii* in the absorption of Mn from heaps of mining enterprises has been described [34]. The maximum element concentration in the leaves of the studied species of woody plants was characteristic for *Acer platanoides*, *Betula pendula*, *Tilia cordata* (180–340 mg/kg), and *Cotoneaster lucidus* (336 mg/kg dry weight). When the concentration of elements in soil exceeds maximum permissible level (MPL) by a factor of 4.7 (site affected by metallurgical enterprises), the contents of the element in the leaves of *Acer platanoides*, *Tilia cordata*, and *Betula pendula* increased by 2–7 times, but not as significantly as in *Aesculus hippocastanum*.

Thus, among the studied species, the maximum accumulation of Mn in the leaves is characteristic of *Acer platanoides*, *Betula pendula*, and *Cotoneaster lucidus*, which is a peculiarity of species.

Table 5.5 Content of heavy metals in the leaves of woody plants, mg/kg of dry weight

Element		**Mn**	**Fe**	**Ni**	**Cu**	**Zn**	**Cd**	**Pb**
RP (Markert [148])/terrestrial vegetation (Kabata-Pendias [149])		**200**	**150**	**1.5**	**10**	**50**	**0.05**	**1**
		15–500	18–1700	0.1–3.7	5–30	1.2–73	0.08–028	0.1–10
Woody plants (zone of metallurgical enterprises and ore dumps) *Acer rubrum*, *Betula pendula*, *Pinus sylvestris*, *Picea*, Lukina, Nikonov [90] Chernenkova [94], Kulagin, Shagieva [34]		197–1055; 157–1050; 195–1130	88–698; 2140–4790; 1630–3680	14–98; 4.5–16; 0.9–11	16–37; 5.1–57; 9.3–26	19–54; 5.6–56; 93	0.14–0.64; 0.42–0.97	1.2–16.3
Acer platanoides	I (KME)	180±22	810±7	0.7±0.3	5.7±0.4	31±6	0.09±0.01	2.6±0.2
	II (Tulachermet)	260±28	1250±41	3.2±0.1	10.1±0.9	44±8	0.16±0.02	0.6±0.1
	Control zone	120±17	310±16	2.2±0.2	8.2±0.7	38±7	0.11±0.02	0.6±0.1
Aesculus hippocastanum	I	53±16	1065±54	4.5±0.7	15.4±1.3	41±9	<0.04	5.6±0.2
	II	91±11	1388±78	1.5±0.2	12.8±0.8	33±8	<0.04	0.6±0.1
	Control zone	83±14	230±12	1.1±0.2	10.7±0.8	32±5	<0.04	0.6±0.1
Betula pendula	I	240±19	800±28	4.3±0.2	6.3±0.5	156±24	0.15±0.02	5.6±0.2
	II	340±7	940±85	6.1±0.4	6.8±0.4	99±12	0.14±0.03	0.5±0.1
	Control zone	45±8	180±24	3.1±0.2	4.3±0.2	53±8	<0.04	0.5±0.1
Populus	I	190±21	1580±62	5.0±0.3	6.8±0.5	171±19	0.58±0.03	8.1±0.2
	II	92±17	500±27	7.2±0.4	10.9±0.9	127±16	0.66±0.04	<0.5
	Control zone	27±5	195±14	1.5±0.1	4.6±0.2	124±18	0.27±0.02	<0.5
Sorbus aucuparia	I	110±16	1780±54	1.2±0.1	6.2±0.4	29±4	0.08±0.01	6.9±0.2
	II	120±14	2570±78	1.8±0.2	6.0±0.3	29±4	0.04±0.01	<0.5
	Control zone	51±7	550±24	1.5±0.1	8.4±0.7	29±3	0.10±0.01	0.9±0.1
Tilia cordata	I	306±54	5082±72	3.5±0.2	7.6±0.6	35±3	0.09±0.01	1.1±0.1
	II	130±14	970±35	1.8±0.1	8.0±0.5	41±3	0.08±0.01	<0.5
	Control zone	130±15	220±25	2.3±0.3	9.0±0.6	41±4	0.10±0.01	0.7±0.1
Larix sibirica	I	110±17	1210±64	0.9±0.1	6.0±0.4	33±2	0.09±0.01	15.2±0.3
	II	120±32	3640±126	4.2±0.4	5.1±0.4	42±4	<0.04	1.6±0.1
	Control zone	410±38	840±32	3.1±0.4	6.5±0.5	41±3	0.06±0.01	1.9±0.2
Excess concentration (toxic concentration) (Cabata-Pendias, 1989)		**≥500**	**≥500; >1000**	**10–100**	**20–40**	**≥300**		**≥60**
Normal concentration (range of normal regulation) (Cabata-Pendias, 1989)		**20–60**	**19–250**	**0.1–2.1**	**3–12**	**20–60**		

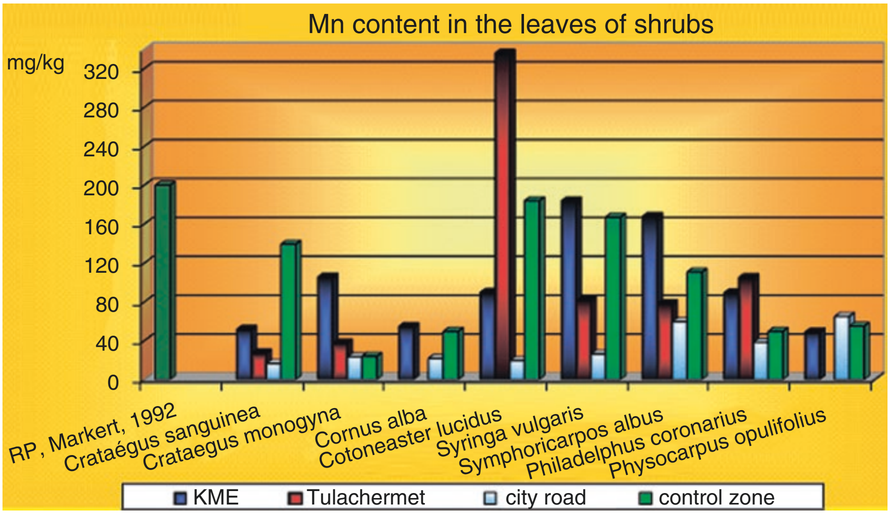

Fig. 5.12 Comparison of Mn content in the leaves of shrubs growing near metallurgical enterprises and city road with RP

5.4.1.2 Iron

The results of investigations showed that the Fe content in leaves of woody plants in urban conditions, in the absence of additional sources of pollution (emissions of enterprises), varies from 48 to 2062 mg/kg of dry weight. In the absence of additional pollution from industries, the minimum iron content is characteristic for leaves of *Populus nigra* (48–195 mg/kg of dry weight) (Table 5.5). The maximum from trees was observed in *Larix sibirica* needles (840 mg/kg) and *Cotoneaster lucidus* leaves (687 mg/kg of dry weight). The content of Fe in the leaves of *Aesculus hippocastanum* and *Tilia cordata* is characterized as close to the average of 142–290 mg/kg, which exceeds the average values for the reference plant [148]. The leaves of *Betula pendula* are characterized by a minimum range of Fe concentrations (154–180 mg/kg).

Moreover, the maximum content of Fe was observed in leaves of *Larix sibirica*, *Sorbus aucuparia*, *Tilia cordata*, *Cornus alba*, and *Cotoneaster lucidus* (Table 5.5, Fig. 5.13).

In general, *Larix sibirica*, *Sorbus aucuparia*, *Tilia cordata*, *Aesculus hippocastanum*, *Acer platanoides*, *Populus nigra*, *Cotoneaster lucidus*, *Cornus alba*, and *Crataegus sanguina* can be considered the best bioaccumulators of Fe when its content in soil is high. The content of Fe in the leaves of these woody species growing in industrial areas is 1250–8930 mg/kg, that is, 10–60 times higher than the values for the reference plant (Table 5.5).

According to some authors, organ concentrators of iron are the roots and bark of trees growing in dumps of polymetallic deposits [94]. The content of Fe in the organs

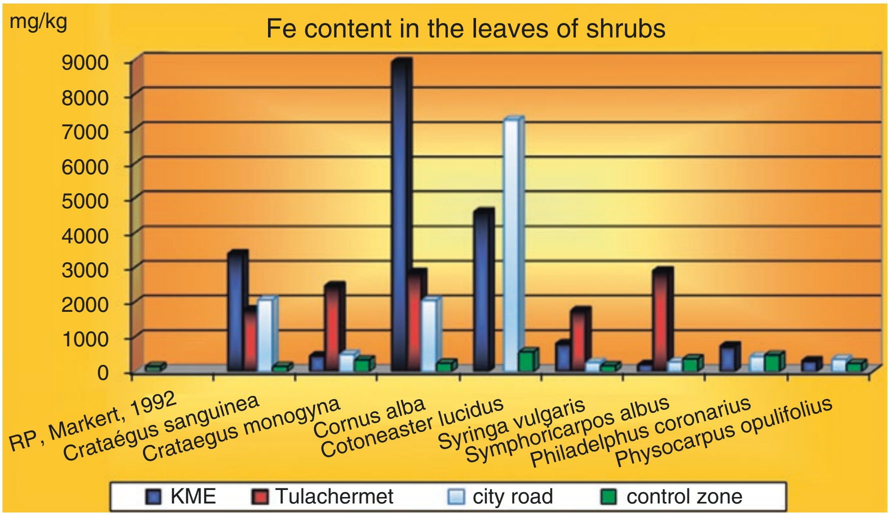

Fig. 5.13 Comparison of Fe content in the leaves of shrubs growing near metallurgical enterprises and city road with RP

(*Betula, Pinus*) amounts to some thousand ppm [34, 90, 94]. Thus, one should expect that the total accumulative ability of trees in the industrial site in relation to iron would be even higher due to accumulation of iron in the perennial organs.

The ratio of Fe/Mn, apparently, is a decisive factor of plant resistance to toxic elements [149]. In this connection, the establishment of relationships in the intake of a given pair of elements in different plant species is of interest. The results obtained show that the ratio of pairs of elements in the accumulation of leaves of woody plants has species peculiarities. Thus, the lowest ratio of Fe/Mn is characteristic for leaves of *A. platanoides*, 0.5–2.5; *Populus nigra* in the Balkan countries, 1–1.4 [98, 99]; *Betula pendula*, 1–4; and *Syringa vulgaris* and *Crataegus sanguina*, 1–1.1 (in buffer zone conditions). A high content of iron relative to manganese is specific for *Aesculus hippocastanum*, 2.5–7; *Tilia cordata*, 2–12; and all other species of investigated shrubs, 3–14. Under the impact of polymetallic contamination, the ratio increases by up to 5 in *Acer platanoides*, 7–16 in *Tilia cordata*, 8 in *Populus nigra*, 16–21 in *Sorbus aucuparia*, 11–30 in *Larix sibirica*, 65 in *Crataegus sanguine*, 9–165 in *Cornus alba*, and 52 in *Cotoneaster lucidus* leaves. There species should be preferably used for phytoremediation of soils from an excess of Fe.

5.4.1.3 Zinc

The concentration of Zn in leaves of woody plants in urban ecosystems is ranging from 16 to 175 mg/kg (Table 5.5, Fig. 5.14). The minimum content of the element in the leaves is characteristic for *Aesculus hippocastanum*, 16–32 mg/kg; Tilia

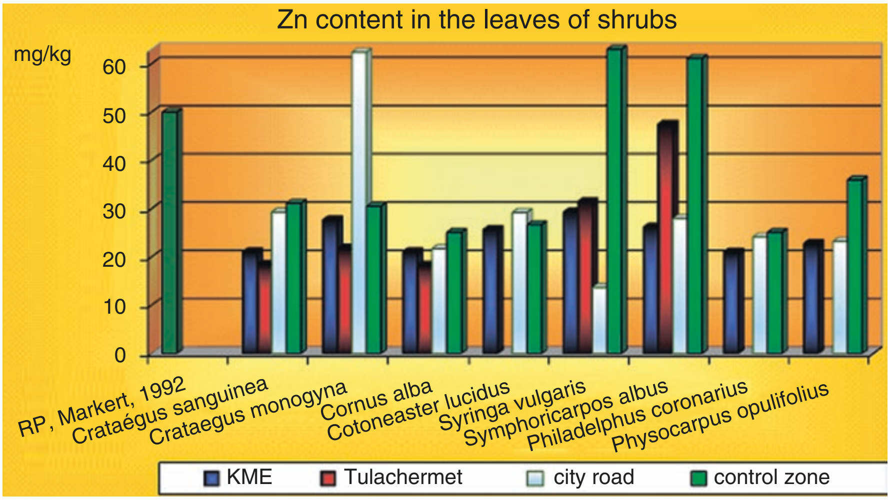

Fig. 5.14 Comparison of Zn content in the leaves of shrubs growing near metallurgical enterprises and city road with RP

cordata, 19–41 mg/kg; and *Crataegus sanguina*, *Cornus alba*, and *Cotoneaster lucidus*, 18–31 mg/kg. Close values of element contents in the leaves are also characteristic for *A. platanoides*. All seven species exhibit a relative resistance with increasing content of Zn in the environment (site of emissions of metallurgical enterprises) that may be due to the known antagonism between the Fe and Zn ions. Data on the antagonism intake of Fe and Zn in the plant organisms [149] are not confirmed for all types of woody plants.

For example, in *A. platanoides*, *Aesculus hippocastanum*, and *Tilia cordata*, increase in accumulation of iron in the leaves takes place along with even a slight accumulation of zinc, i.e., iron absorption dominates over the absorption of zinc.

However, for the species accumulators of Zn, *Betula pendula* and *Populus nigra*, an increased concentration of all three element antagonists Fe, Mn, and Zn—in the site of polymetallic pollution—was revealed. The concentration of Zn increased up to 153–176 mg/kg that exceeds the average for terrestrial plants by a factor of 3–3.5. Such undiscriminating absorption of three elements at their high concentrations in the soil by the given species may be due to the absence of barrier function of the root system to absorption of Fe, Mn, and Zn that is a characteristic peculiarity of hyperaccumulators [29].

5.4.1.4 Nickel

Accumulation of Ni by leaves of woody plants was low and ranged from 0.1 to 4.5 mg/kg of dry weight within urban ecosystems (Table 5.5, Fig. 5.15). The values of Ni content in the leaves of trees exeeds average values for the reference plants by a factor of 1.5–2 of the species *Acer platanoides, Betula pendula, Tilia cordata,* and *Larix sibirica.* However, in the site affected by the metallurgical

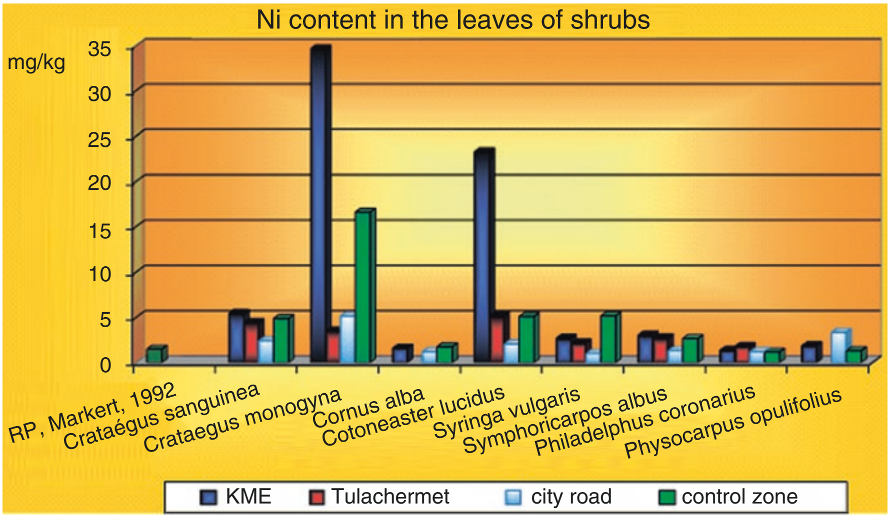

Fig. 5.15 Comparison of Ni content in the leaves of shrubs growing near metallurgical enterprises and city road with RP

industry, the content of the element in the leaves of trees increased by a factor of 1.5–2 for the species *Acer platanoides*, *Tilia cordata*, and *Betula pendula* and more than a factor of 4–4.5 for *Aesculus hippocastanum* and *Populus nigra* (up to 4.3–7.2 mg/kg), exceeding the values for the reference plants by 3–5 times. High concentrations of nickel were found for shrubs *Crataegus*, *Cotoneaster lucidus*, and *Syringa vulgaris* in control zone with 4.9–16.6 mg/kg of dry weight. In the sampling sites affected by the metallurgical industry, the content of the element in the leaves of shrubs increased by a factor of 2–5 for such shrubs as *Crataegus monogina* (KME) and *Cotoneaster lucidus.* However, the total concentration of nickel in the tree leaves did not exceed the threshold of phytotoxicity of the element and was located in the middle of toxic concentrations for species of shrubs such as *Crataegus monogyna* and *Cotoneaster lucidus*. High concentration of heavy metals (nickel in particular) can cause necrosis of *Crataegus* leaves in case of low activity of antioxidant system [149].

5.4.1.5 Lead

Accumulation of Pb by leaves of studied trees and shrubs was low and ranged within 0.5–2.7 mg/kg (Table 5.5, Fig. 5.16). In the areas affected by emissions of metallurgical enterprises, when the concentration of the element in soil exceeds the maximum permissible level, *Tilia cordata* showed the greatest resistance, the concentration of elements in the leaves of which increased slightly compared with the relatively clean area. Possible low bioaccumulation of lead by *Tilia* leaves is due to the peculiarities of leaf surface. However, in the leaves of *Aesculus hippocastanum*, *Betula pendula*, *Populus nigra*, *Sorbus aucuparia*, *Larix sibirica* (sample point KME), *Philadelphus coronarius*, and *Cotoneaster lucidus* (sample point Tulachermet), the

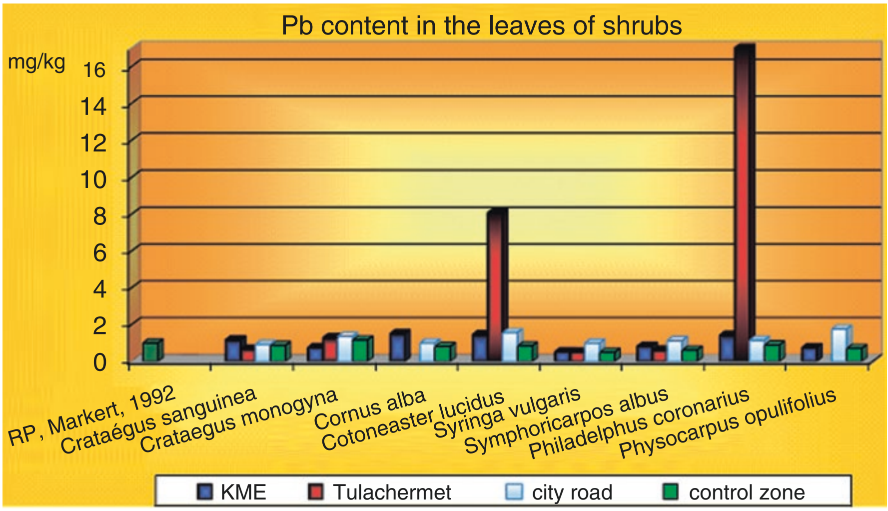

Fig. 5.16 Comparison of Pb content in the leaves of shrubs growing near metallurgical enterprises and city road with RP

concentration of element in the leaves in the impact site of metallurgical enterprises increased by a factor of 9–16 and reached the values of 5.6–17 mg/kg, which is higher than in the “reference plant” by a factor of 5–17. However, in our case, the general element concentrations in leaves of most woody plants do not exceed the values for herbaceous terrestrial plants growing in nonmetallic areas (1–10 mg/kg) [149]. Only for *Larix sibirica* and *Philadelphus coronarius* grown under conditions of metallurgical enterprises, the value of lead concentrations in the leaves is above the average values (15–17 mg/kg). It is known that the transport of lead in the roots is passive, and the major part of the element is kept at a certain level in roots and in the tissue accumulators (inner and outer cortex) [29], but in its sufficient quantity, it can be absorbed by leaves from the air [149]. This is probably the main way of absorption and accumulation of lead by leaves of woody plants [173]. This confirms the fact of the higher lead content in the tree leaves of the cities of the Balkan countries, where sampling is performed in urban heavy traffic areas in comparison with the parks in Russia. For example, high concentration of many metals and toxic elements in dust in Sofia was observed, e.g., up to 192 mg/kg of Pb, 8 mg/kg of As, 123 mg/kg of Cu, 710 mg/kg of Zn, etc. [98].

It was established that leaves of woody plants are suitable for biomonitoring of elements in urban environments, and in the Mediterranean for that purpose such species as *Quercus ilex* was used [2, 3, 88, 174]. However, as it was demonstrated in our study, most of the examined species of trees and shrubs react to the polymetallic pollution accumulating some of these elements.

Of all studied species, only in *Aesculus hippocastanum*, *Betula pendula*, *Tilia cordata*, *Populus nigra*, *Crataegus sanguine* and *monogina*, and *Cornus alba*, combined effect of polymetallic contamination with Mn, Fe, Ni, Pb, and Zn causes stable morphological changes, namely, decrease of vitality, appearance of necrosis, and chlorosis of leaves (Figs. 5.8 and 5.10; Table 5.6).

Table 5.6 The possibility of using the studied species of woody plants for bioremediation of soils contaminated with heavy metals (Gorelova et al. [98, 99, 101, 102, 145, 175])

Species	Vitality/ steadiness to polymetallic pollution	Morphological changes	Ability to bioaccumulate heavy metals under the influence of polymetallic contamination, mg/kg/concentration factor in relation to the reference plants
Aesculus hippocastanum	2–3/–, the death of up to 30% of trees at planting	Regional and interveinal necrosis of leaves	Fe—970–1388/6.5–9 Ni—4.5/3 Cu—12.8–15.4/1.3–1.5 Pb—5.6 /5
Betula pendula	1–2/+	Point necrosis of leaves	Mn—240–340/1.2–1.7 Fe—800–940/5–6 Ni—4.3–6.1/3–4 Zn—99–156/2–3 Pb—5.6/5
Populus nigra	3/–	Necrosis of leaves, dry branches in the crown, dieback	Fe—500–1580/3–11 Ni—5–7.2/3–5 Zn—127–171/2.5–3.4 Cd—0.58–0.66/11–13 Pb—8.1/8
Sorbus aucuparia	2/+	Necrosis of the the leaf margin, chloroses	Fe—1780–2570/11–17 Pb—6.9/7
Larix sibirica	1/+	–	Fe—1210–3640/8–24
Crataegus sanguinea	2/+ in the absence of pruning	25–28% necrotic spots on the leaves	Cl—1720–2560/2 V—2.9–5.5/6–11 Fe—1780–3400/12–23 Ni—4.5–5.5/3–3.7 Cu—26/2.5
Crataegus monogyna	2/+ in the absence of pruning	10–37% regional and interveinal necrosis and chloroses of leaves	V—4.7/9 Fe—457–2470/3–16 Ni—16.6–34.7/11–23 Cu—14/1.4
Cornus alba	2/+ depends on the emissions components	24–28% necrosis of the the leaf margin, chloroses, pest insect damage (aphid)	Cr—6.5/4 Fe—2050–8930/14–59
Cotoneaster lucidus	1–2/+	9% regional and interveinal necrosis of leaves, chloroses	Mn—336/1.5 Fe—2860–7260/19–48 Ni—5–23/3–15 Cd—0.123/2.5 Pb—1.4–8.1/1.4–8
Symphoricarpos albus	1–2/+	4–5% point necrosis of leaves	V—4.4/9 Cr—4.2–5.6/3–3.5
Syringa vulgaris	1/+	4–5% necrosis of the the leaf margin	Cl—4460/22 V—3/6 Cr—5.2/3.4 Fe—810–2310/5–15
Philadelphus coronarius	1/+	7%—necrosis of the the leaf margin, chloroses	Cl—4830/24 V—5.3/10 Fe—430–735/3–5 Pb—1.4–17/1.4–17

This fact demonstrates preference of using the given species for bioindication. It is known that the meaning of bioindicator and biomonitor is not identical [148]. Due to this fact, the choice of biomonitors should be based on the whole set of features. The recommended biomonitors in heavy metal pollution areas are *Populus nigra*, *Betula pendula*, and *Cotoneaster lucidus* as concentrations of all studied elements in polymetallic pollution increase sharply in these species (Table 5.6). The other species can be used for biomonitoring selectively, given the species specificity of the absorption of elements.

On the contrary, for phytoremediation it is more reasonable to use species which have directed adaptive changes and which preserve normal vitality in the conditions of polymetallic contamination.

The number of accumulating elements in this case diminishes, but duration of detoxication of the environment increases. According to the results obtained, *Betula pendula*, *Sorbus aucuparia*, *Tilia cordata* and *Larix sibirica*, and *Cotoneaster lucidus* belong to such species.

5.4.2 *Transfer Factor of Elements in Trees and Shrub Leaves from the Soil*

Heavy metals enter plants in two ways: by absorbing root system and by uptake through the aboveground organs. An objective criterion, which characterizes the efficiency of accumulation of chemical elements, is the factor of biological accumulation or transfer factor (TF) [176]. To identify possible sources of contamination, it is also important to know the level of air pollution [177]. Part of pollutants are rather hygroscopic and can penetrate the epidermis and stomata in the form of tiny particles as well as a concentrated solution, causing water shortages and promoting early defoliation [178].

The results of the calculation of transfer factor (TF) for woody plants in the buffer site of metallurgical enterprises are shown in Tables 5.7 and 5.8. For most heavy metals, the values of TF are within 0.01–0.2. However, for some species, specificity of accumulation of elements in leaves relatively their concentration in the soil was observed. For example, for *Populus* leaves known as accumulator of heavy metals, the TF for Cd > 1 and it is 5–15 times higher than for other species. These values of TF may be associated with foliar absorption. Poplar leaves secrete sticky substances that promote the transition of insoluble compounds of aerosol particles in the soluble forms of active transport in the leaves cells [94]. However, under the increase of Cd content in the soil, we observed decrease of TF in five times (sampling point I), which is clearly associated with increased barrier function of the root system. Because of bioaccumulation from the soil, the content of Zn in the *Populus* species was higher than its total content in the soil. However, when Zn concentration exceeded the maximal permissible level in the soil by two times, as well as in the case of cadmium, the TF decreased due to barrier function of the roots.

Table 5.7 TF of woody plant leaves in buffer site of metallurgical enterprises (Tula, Russia)

Species	Sampling point	Mn	Fe	Ni	Zn	Cd	Pb
Acer platanoides	I	0.03	0.06	0.01	0.13	0.08	0.03
	II	0.29	0.03	0.08	0.35	0.62	0.02
	Control point	0.16	0.03	0.08	0.58	0.65	0.02
Aesculus hippocastanum	I	0.01	0.06	0.10	0.17	0.03	0.06
	II	0.10	0.02	0.04	0.26	0.15	0.02
	Control point	0.11	0.02	0.04	0.49	0.24	0.02
Betula pendula	I	0.03	0.05	0.09	0.66	0.13	0.06
	II	0.37	0.03	0.16	0.79	0.54	0.02
	Control point	0.06	0.02	0.11	0.82	0.24	0.02
Populus nigra	I	0.03	0.11	0.11	0.73	0.50	0.09
	II	0.10	0.01	0.19	1.01	2.54	0.02
	Control point	0.03	0.02	0.05	1.91	1.59	0.02
Tilia cordata	I	0.01	0.07	0.07	0.15	0.08	0.01
	II	0.14	0.03	0.05	0.33	0.31	0.02
	Control point	0.17	0.02	0.08	0.63	0.59	0.02

Table 5.8 TF of shrub leaves in buffer site of metallurgical enterprises (Tula, Russia)

Species	Samling point	Mn	Fe	Ni	Zn	Cu	Cd	Pb
Symphoricarpos albus	Control point	0.14	0.04	0.09	0.94	0.22	0.0013	0.019
	I	0.02	0.01	0.09	0.11	0.10	0.0002	0.009
	II	0.09	0.08	0.07	0.38	0.11	0.0002	0.024
Syringa vulgaris	Control point	0.22	0.02	0.18	0.98	0.37	0.0018	0.015
	I	0.03	0.06	0.08	0.12	0.15	0.0007	0.006
	II	0.09	0.05	0.06	0.25	0.19	0.0004	0.020
Cotoneaster lucidus	Control point	0.24	0.06	0.18	0.41	0.31	0.0013	0.026
	I	0.01	0.32	0.65	0.06	0.10	0.0003	0.016
Philadelphus coronarius	Control point	0.06	0.05	0.06	0.39	0.27	0.0003	0.028
	I	0.01	0.05	0.04	0.09	0.10	0.0002	0.015
	II	0.12	0.07	0.05	0.11	0.28	0.0003	0.652
Crataegus monogina	Control point	0.03	0.04	0.59	0.47	0.21	0.0012	0.037
	I	0.01	0.23	0.97	0.09	0.30	0.0005	0.008
	II	0.04	0.07	0.09	0.17	0.12	0.0005	0.049
*Crataegus sanguine*a	Control point	0.18	0.02	0.17	0.48	0.25	0.0014	0.027
	I	0.01	0.01	0.15	0.21	0.17	0.0004	0.013
	II	0.03	0.05	0.12	0.15	0.68	0.0001	0.026
Cornus alba	Control point	0.06	0.02	0.06	0.39	0.20	0.0003	0.025
	I	0.01	0.61	0.04	0.09	0.11	0.0002	0.016
	II	0.01	0.08	0.04	0.15	0.09	0.0002	0.016
Physocarpus opulifolius	Control point	0.07	0.02	0.05	0.55	0.30	0.0014	0.022
	I	0.01	0.02	0.05	0.10	0.15	0.0015	0.008

A reduction of the TF, while excess of the maximal permissible levels (MPL) in the soil of the total content of Ni, Zn, and Cd for *A. platanoides*; of Mn, Zn, and Cd for *Aesculus hippocastanum*; and of Mn, Zn, Cd, and Pb for *Tilia cordata*, is also observed (Table 5.7). For phytoextraction of heavy metals from soils, most advisable species are those in which the barrier mechanisms are not working at high concentrations of elements in the environment; however, the mechanism of physiological adaptation and TF increases compared to the background site. When selecting plants for phytoremediation, it is important to consider also species specificity in the accumulation of individual elements. The results obtained showed that with increasing Fe concentration in the medium in all species studied increased the value of TF for the transport from soil to leaves (Tables 5.5 and 5.6). For the sampling site (I), an increase in the value of TF for Fe may be due to foliar absorption element from aerosol particles (the concentration of elements in the air increases). However, due to the fact that iron is an element, concentrated mainly in the roots [108], for all kinds of woody plants, TF values for Fe would be considered low compared with the values for Cd and Zn, due to the barrier function of endoderm and low transport capacity of elements in the acropetal direction.

The average TF for investigated woody plants is as follows:

Mn—0.01…0.37 and maximal for *Betula pendula*
Fe—0.01…0.61, maximal for *Cornus alba*
Ni—0.04…0.18, but for *Crataegus monogyna* 0.97
Zn—0.06…0.48, maximally for *Cornus alba*
Cu—0.09…0.68, maximally for *Crataegus sanguinea*
Cd—0.0002…0.0018
Pb—0.009…0.049 but for *Phyladelphus coronarius* 0.65

For the species which TF of elements is greater than 0.2 (Tab. 5.7, 5.8) the bioremediation of soils from heavy metals will last for 2–10 years (depending on leaves biomass formed during the growing season, water content in leaves, nutrition areas, and the species values of TF) in the absence of additional receipt of heavy metals from the environment.

5.5 Statistical Analysis of the Results

One of the methods to identify the main trends in the intake of elements from the environment is multivariate statistical analysis. We carried out correlation, cluster, and factor analyses of the results of the study, which allowed to identify the main elements—environmental pollutants and their groups. Figure 5.17 shows the dendrogram of groups of elements, which woody plants bioaccumulated from the environment.

Results of cluster analysis clearly distinguished group of elements, which can be divided into several categories:

Group 1: Ca-K are elements-antagonists which play an important role in creating an osmotic pressure in plant cells and the regulation of processes in the plant.

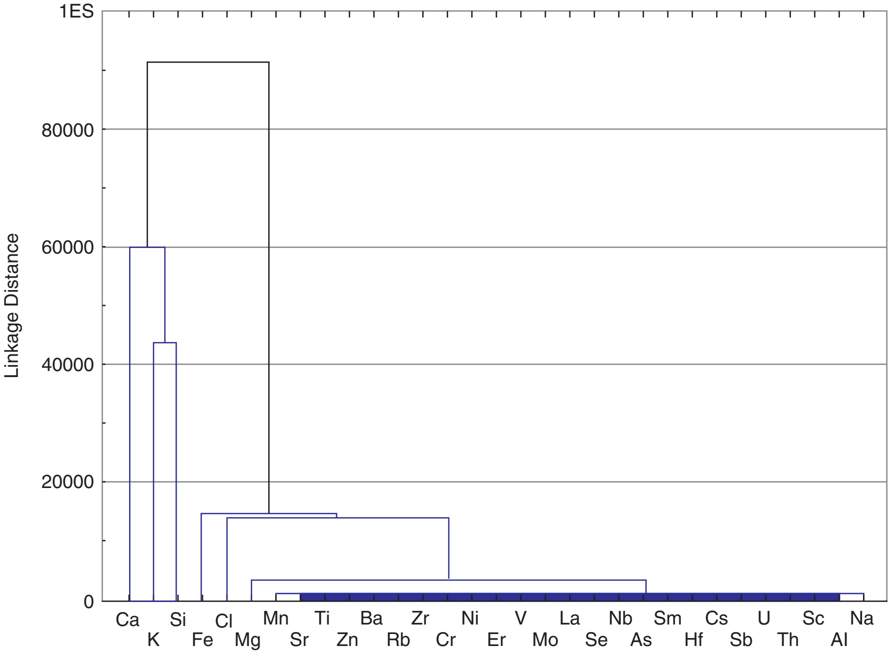

Fig. 5.17 Cluster analysis of bioaccumulation of the elements from the polluted environment by woody plants

Group 2: K-Si are biologically essential elements. Si can be included in the composition of cell walls of plants.

Group 3: Combined elements are organogenic (essential elements) Ca, K, Si, Fe, Cl, and Mg.

Group 4: Combined elements—environmental contaminants of the region Fe, Cl, Mn, Sr, and Ti, associated with soil and ferrous metallurgy.

Group 5: Soil elements and pollutants associated with the processing of ores.

Group 6: The components of the metallurgical and defense industry that can be combined into a conglomerate entering the plant, including root uptake from soil resuspention and atmospheric deposition.

Correlation analysis (Table 5.9) of bioaccumulation of elements by woody plants growing along highways of industrially contaminated city reflects the bioaccumulation characteristics of plants and components of the environmental pollution from the activity of enterprises, highlighting element group and links between them.

Ca-Mg are divalent essential elements with the same way of transport play an important role in the life of plants (Mg is a component of chlorophyll, the regulator of photosynthesis processes, Ca is a regulator of cellular immunity, enzymatic processes in the cell, component of microtubules and is a cell wall component).

Elements that can substitute each other in the biochemical processes in plants are K-Rb and Ca-Sr.

Elements associated with the soil particles are Na-Mg, Na-Al, and Ca-Al.

Table 5.9 Correlation analysis of the element bioaccumulation in woody plants growing near highways

	Na	Mg	Al	K	Ca	Sc	V	Cr	Mn	Fe	Ni	Co	Cu	Zn	As	Se	Rb	Sr	Zr	Mo	Ag	Cd	Nd	Sm	Eu	Tb	Yb	Hf	Ta	W	Hg	Th	U
Na	1.00																																
Mg	0.56	1.00																															
Al	0.74	0.63	1.00																														
K	0.47	0.46	0.37	1.00																													
Ca	0.48	0.74	0.57	0.35	1.00																												
Sc	0.16	0.05	0.30	0.31	0.13	1.00																											
V	0.42	0.34	0.72	0.05	0.34	0.14	1.00																										
Cr	−0.15	−0.16	0.08	−0.04	−0.24	0.26	0.17	1.00																									
Mn	0.09	0.34	0.32	0.02	0.32	0.12	0.52	−0.07	1.00																								
Fe	0.45	0.52	0.82	0.29	0.44	0.31	0.78	0.23	0.53	1.00																							
Ni	−0.21	−0.23	−0.02	−0.07	−0.28	0.04	0.17	0.86	−0.06	0.17	1.00																						
Co	0.17	0.11	0.31	0.05	0.06	0.49	0.27	0.62	0.08	0.30	0.28	1.00																					
Cu	0.19	0.13	0.12	0.21	0.24	−0.01	0.01	−0.23	0.16	0.00	−0.27	0.09	1.00																				
Zn	0.36	0.14	0.14	0.09	0.11	−0.14	0.00	−0.42	0.14	0.01	−0.40	−0.22	0.25	1.00																			
As	0.36	0.37	0.26	0.27	0.38	−0.08	0.07	−0.68	0.12	0.13	−0.58	−0.44	0.11	0.35	1.00																		
Se	0.09	0.12	0.27	0.19	0.07	0.62	0.17	0.54	0.03	0.31	0.28	0.59	−0.08	−0.14	−0.23	1.00																	
Rb	0.26	0.29	0.17	0.65	0.19	0.17	−0.08	−0.12	−0.12	0.12	−0.11	−0.08	0.19	−0.01	0.30	0.13	1.00																
Sr	0.49	0.45	0.51	0.29	0.68	0.14	0.31	−0.32	0.13	0.36	−0.33	−0.12	0.07	0.16	0.54	0.17	0.44	1.00															
Zr	0.01	0.01	0.19	0.17	−0.06	0.54	0.12	0.74	−0.05	0.25	0.35	0.84	0.06	−0.34	−0.50	0.68	0.04	−0.17	1.00														
Mo	0.06	0.01	0.03	−0.06	0.07	−0.11	−0.06	−0.16	0.15	−0.03	−0.14	−0.13	0.02	0.00	0.03	−0.11	−0.06	0.05	−0.15	1.00													
Ag	−0.06	−0.03	0.12	0.07	−0.14	0.47	0.09	0.76	−0.04	0.20	0.40	0.78	−0.01	−0.20	−0.56	0.72	−0.06	−0.24	0.92	−0.18	1.00												
Cd	0.11	0.24	0.28	0.34	0.18	0.37	0.22	0.54	0.10	0.29	0.28	0.61	0.07	−0.16	−0.43	0.55	0.09	−0.05	0.72	−0.11	0.76	1.00											
Nd	−0.04	0.19	0.16	0.13	0.06	0.39	0.06	0.36	0.14	0.21	0.08	0.46	0.03	0.20	−0.19	0.66	0.01	−0.03	0.57	−0.14	0.74	0.65	1.00										
Sm	0.36	0.44	0.68	0.24	0.47	0.37	0.64	−0.08	0.57	0.77	−0.13	0.13	0.07	−0.08	0.27	0.17	0.14	0.46	0.07	−0.02	−0.02	0.19	0.10	1.00									
Eu	−0.23	−0.10	−0.10	0.18	0.01	0.58	−0.06	0.17	−0.01	−0.02	0.10	0.20	−0.12	−0.32	−0.01	0.57	0.25	0.20	0.32	−0.17	0.34	0.33	0.40	0.22	1.00								
Tb	−0.06	0.10	0.15	0.02	0.11	0.30	0.11	0.04	0.29	0.23	−0.09	0.24	0.02	0.33	−0.04	0.42	−0.08	0.03	0.20	−0.12	0.43	0.39	0.76	0.19	0.30	1.00							
Yb	−0.23	−0.13	−0.03	0.02	−0.22	0.31	0.04	0.75	−0.07	0.09	0.64	0.46	−0.21	−0.14	−0.48	0.64	−0.08	−0.27	0.60	−0.22	0.78	0.62	0.66	−0.13	0.49	0.46	1.00						
Hf	−0.06	0.00	0.14	0.04	−0.11	0.44	0.09	0.76	−0.05	0.21	0.39	0.79	−0.02	−0.21	−0.57	0.67	−0.12	−0.28	0.94	−0.15	0.98	0.75	0.72	−0.04	0.25	0.38	0.73	1.00					
Ta	−0.18	0.00	−0.10	0.06	−0.07	0.19	−0.06	0.15	0.03	−0.04	0.03	0.23	−0.02	0.21	−0.17	0.43	−0.06	−0.15	0.33	−0.17	0.55	0.51	0.77	−0.09	0.45	0.71	0.68	0.50	1.00				
W	0.24	0.25	0.42	0.22	0.32	0.24	0.34	0.18	0.20	0.39	0.07	0.33	0.05	0.07	0.07	0.48	0.19	0.40	0.31	0.00	0.35	0.46	0.49	0.41	0.34	0.40	0.33	0.29	0.35	1.00			
Hg	−0.02	0.07	0.11	0.23	0.10	0.53	0.01	0.22	−0.08	0.14	0.03	0.32	−0.05	−0.29	0.11	0.54	0.27	0.29	0.51	−0.31	0.42	0.30	0.35	0.26	0.69	0.13	0.38	0.39	0.31	0.34	1.00		
Th	0.55	0.50	0.80	0.34	0.39	0.47	0.57	0.34	0.31	0.78	0.14	0.46	−0.09	0.08	0.06	0.54	0.14	0.35	0.44	−0.04	0.44	0.44	0.45	0.60	0.08	0.32	0.25	0.43	0.09	0.51	0.20	1.00	
U	0.65	0.50	0.81	0.30	0.38	0.36	0.65	0.11	0.24	0.63	0.00	0.36	0.08	0.05	0.24	0.33	0.15	0.41	0.23	−0.02	0.18	0.31	0.19	0.53	0.03	0.11	0.03	0.18	−0.04	0.39	0.18	0.72	1.00

Contaminants of city soil and particulate emissions into the atmosphere from components of metallurgical production and processing of ore are Fe-V, Fe-Mn, Cr-Ni, Cr-Co, and Sr-As.

Groups with a high correlation between heavy metals and rare earth element-pollutants of the atmosphere and soil of the city, originate from the sources of plant pollution—enterprises of the defense industry, instrumentation, and metallurgy.

Multivariate statistical analysis revealed three factors (Table 5.10):

Factor 1 is associated with ores used for production of steel and alloys.

Factor 2 can be attributed to technogenic pollution (metallurgical production) and soil particles.

Factor 3 is associated with physiological activity of plants.

Table 5.10 Factor analysis of elements bioaccumulation by woody plants

	Factor 1	Factor 2	Factor 3
Na	−0.14	0.73	0.11
Mg	−0.08	0.72	0.30
Al	0.11	0.92	0.05
S	−0.13	0.17	0.04
Cl	0.15	0.29	0.32
K	0.05	0.35	0.54
Ca	−0.16	0.67	0.34
Sc	0.53	0.22	0.34
Ti	−0.12	0.44	0.00
V	0.13	0.77	−0.21
Cr	0.83	0.00	−0.37
Mn	−0.03	0.56	−0.01
Fe	0.23	0.85	−0.05
Ni	0.53	−0.07	−0.44
Co	0.78	0.27	−0.11
Cu	−0.13	0.14	0.25
Zn	−0.31	0.20	0.13
As	−0.60	0.29	0.49
Se	0.77	0.17	0.27
Br	−0.03	0.30	0.62
Rb	−0.07	0.12	0.60
Sr	−0.24	0.51	0.47
Zr	0.90	0.10	0.03
Mo	−0.21	0.05	−0.14
Ag	0.96	0.03	0.02
Cd	0.76	0.23	0.21
In	−0.28	0.16	0.16
Sb	−0.14	0.56	0.21
I	−0.09	0.64	0.04

(continued)

Table 5.10 (continued)

	Factor 1	Factor 2	Factor 3
Ba	0.23	0.39	−0.04
Cs	0.54	0.38	0.16
Nd	0.72	0.11	0.34
Sm	0.04	0.72	0.23
Eu	0.46	−0.20	0.62
Tb	0.43	0.13	0.28
Dy	0.08	0.25	0.43
Yb	0.84	−0.15	0.06
Hf	0.94	0.04	−0.04
Ta	0.54	−0.17	0.38
W	0.38	0.38	0.38
Au	−0.04	0.20	0.14
Hg	0.46	−0.02	0.59
Th	0.43	0.77	0.06
U	0.18	0.77	0.07
Expl. var	9.35	8.25	4.12
Prp. totl	0.21	0.19	0.09

5.6 Conclusion

- Woody plants can be good bioindicators of the environmental pollution with heavy metals (air and soil) affecting the morphological parameters: the development of necrosis and leaf chlorosis between 25 and 98% for unresisting species to pollution. For the purposes of bioindication, species of woody plants such as *Populus nigra*, *Tilia cordata*, *Aesculus hippocastanum*, *Cornus alba*, *Crataegus monogina*, and *Crataegus sanguinea* can be used.
- Analysis of the dust particles on the surface of woody plant leaves using method of electron scanning microscopy can be used as the qualitative method for analysis of components of aerosol emissions and allows one to establish the presence and ratio of the components on the leaf surface that reflects the air pollution.
- Woody plant species can be used as biomonitor of technogenic emissions due to their ability to bioaccumulate Cl, V, Mn, Fe, Ni, Zn, Cu, As, Cd, and Pb which characterize the anthropogenic pollution of soil and air (compared to background values or control zone):
- *Acer platanoides*—Cl, V, Mn, Fe, Ni, Cu, As, and Cd
- *Aesculus hippocastanum*—Ni, Cu, As, and Pb
- *Betula pendula*—Mn, Fe, Ni, Zn, Cd, and Pb
- *Cotoneaster lucidus*—Mn, Fe, Ni, Cu, Cd, and Pb
- *Crataegus monogyna*—Fe and Ni
- *Larix sibirica*—Fe and Pb
- *Philadelphus coronarius*—Pb and Sb
- *Populus nigra*—Mn, Fe, Ni, Zn, Cd, and Pb
- *Salix fragilis*—Cl, V, Mn, Ni, Zn, As, and Cd
- *Tilia cordata*—Cl, Mn, Ni, and Cd

Most of the studied species are good biomonitors to study contamination of soil and air with iron.

- Woody plants which form a large biomass of leaves per season are able to absorb heavy metals to the extent exceeding several times the values characteristic for the reference plants. This allows to recommend them for phytoremediation of the environment from heavy metals:
- *Aesculus hippocastanum*—Ni, Cu, As, and Pb
- *Betula pendula*—Mn, Fe, Ni, Zn, Cd, and Pb
- *Crataegus sanguinea* and *C. monogina*—Cl, V, Fe, Ni, and Cu
- *Cornus alba*—Cr and Fe
- *Cotoneaster lucidus*—Mn, Fe, Ni, Cd, and Pb
- *Syringa vulgaris*—Cl, V, Cr, Fe, and Cu
- *Sorbus aucuparia*—Fe and Pb
- *Philadelphus coronarius*—Pb and Sb
- Populus nigra—Fe, Ni, Zn, Cd, and Pb
- *Larix sibirica*—Fe and Pb
- Analysis of element transfer from the soil into the leaf biomass of woody plants (TF) has shown that for a number of elements (Ni, Cd, Zn), increase of their content in the soil leads to decreased transfer of these elements in the leaves of woody plants. That fact could be a sign of the barrier function of the root system.

Relatively high values of TF (0.2–1) for the elements Fe (species *Crataegus sanguinea* and *C. monogina*, *Cotoneaster lucidus*, *Cornus alba)*, Cu (species *Crataegus sanguinea* and *C. monogina*, *Philadelphus coronarius*), Zn (species *Acer platanoides*, *Aesculus hippocastanum*, *Betula pendula*, *Crataegus sanguinea*, *Betula pendula*, *Symphoricarpos albus*, *Syringa vulgaris*, *Populus nigra*), Cd (species *Acer platanoides*, *Betula pendula*, *Populus nigra*, *Tilia cordata*), and Pb (*Philadelphus coronarius*) in woody plants confirm the possibility of their use for phytoremediation from the enlisted elements.

- When selecting plants for phytoremediation, it is important to consider that species of *Aesculus hippocastanum*, *Crataegus* sp., *Cornus alba*, and *Populus nigra* are not resistant to the integrated pollution with heavy metals. Species *Betula pendula*, *Cotoneaster lucidus*, *Syringa vulgaris*, *Philadelphus coronarius*, *Sorbus aucuparia*, and *Larix sibirica* are resistant to high level of pollution (have normal vitality) and can be used in the creation of sanitary-protective zones of metallurgical enterprises and greenbelts.
- Methods of statistical analysis (correlation and factor analysis) in the processing of the biogeochemical composition of leaves of woody plants allow to clearly reveal group of elements polluting the study area.

Acknowledgments *The financial support by the*

- *RFBR grant mob_st. № 09-05-90722 scientific work of the Russian young scientist Svetlana Gorelova in the Geological Institute of Russian Academy of Sciences on the project: "Study of biogeochemical variability of plants under conditions of intense anthropogenic soil pollution by using modern physical and physical-chemical methods of analysis"*

- *The Black Sea Economic Cooperation—Project Development Fund (Research Contract No. BSEC/PDF/0018/11.2008—01.2010) «Revitalization of urban ecosystems through vascular plants: assessment of technogenic pollution impact»). BSEC (Союз Балканских государств) (Болгария, Греция, Россия, Румыния, Сербия, Турция) The Serbian scientific group also acknowledges support by MSTD RS (Contract No. 141012).*
- *RFBR grant r_center_a 13-05-97508 "The study of adaptive characteristics and buffer role of woody exotic species in the migration of toxic elements in the urban ecosystems"*
- *RFBR 13-05-97513 r_center_a "Evaluation of the sustainability of the state and prognosis of natural and anthropogenic ecosystems in Central Russia (Tula Region)"*
- *RFBR grant r_center_a 15-45-03252 "Biomonitoring of air pollution by industrial emissions in forest and forest-steppe ecosystems of the Central regions of Russia (example of Tula region)"*

The authors are grateful

- *for participation in our research, support and fruitful collaboration to the head of the laboratory of chemical and analytical investigations of the Geological Institute of the Russian Academy of Sciences Sergey Lyapunov and Senior Research Scientists Anatoly Gorbunov and Olga Okina;*
- *for collection of plant material in ecosystems Tula region, for participation in our reseach, fruitful discussions and scientific inspiration to the head of the RFBR grant 13-05-97513 r_center_a "Evaluation of the sustainability of the state and prognosis of natural and anthropogenic ecosystems in Central Russia (Tula Region)", Ph.D. of Biological Sciences, Associate Professor of Tula State University and my friend Elena Volkova;*
- *for help in the statistical data treatment to Lecturer of Radiation Ecology, Radiation Protection Expert of Radiation Protection and Civil Defense Department, Nuclear Research Center, Egyptian Atomic Energy Authority, Cairo, Egypt, Wael Badawy;*
- *for participation in the study of soil, air pollution and woody plants to my diploma students (L.N. Tolstoy Tula State Pedagogical University)*

References

1. Markert B, Breure A, Zechmeister HG (eds) (2003) Bioindicators and biomonitors, principles, concepts and applications. Elsevier, Amsterdam, 997 p
2. Bargagli R (1998) Trace elements in terrestrial plants. An ecophysiological approach to biomonitoring and biorecovery. Springer, Berlin, 324 p
3. Bargagli R (2005) Trace elements in terrestrial plants: an ecophysiological approach to biomonitoring and biorecovery. M GEOS:457 (Russian)
4. Wolterbeek B (2002) Biomonitoring of trace element air pollution: principles, possibilities and perspectives. Environ Pollut 120:11–21
5. Wolterbeek B (2003) Biomonitoring of trace element air pollution: principles, possibilities and perspectives. Biomonitoring of atmospheric pollution (with emphasis on trace elements). BioMAP II. IAEA, Vienna, pp 87–105
6. Muneer S, Qardi TN, Mahmooduzaffar N, Siddigqi O (2011) Cytogenetic and biochemical investigations to study the response of *Vigna radiata* to cadmium stress. Afr J Plant Sci 5(3):83–192
7. Lepp NW (ed) (1981) Effect of heavy metal pollution on plants. Effects of trace metals on plant function, vol 1. Applied Science, London
8. Brown DH, Wells JM (1990) Physiological effects of heavy metals on the moss *Rhytidiadelphus squarrosus*. Ann Bot 66:641–647
9. Kenya M, Lukasz AI, Guskov EP (1993) Role of low molecular antitioksidants under oxidative stress. Success lies. Biology 113(4):456–470 (Russian)
10. Wu F-B, Chen F, Wei K, Zhang G-P (2004) Effects of cadmium on free amino acid, glutathione and ascorbic acid concentrations in two barley genotypes (*Hordeum vulgare* L.) differing in cadmium tolerance. Chemosphere 57:447–454

11. Bao T, Sun T, Sun L (2011) Low molecular weight organic acids in root exudates and cadmium accumulation in cadmium hyperaccumulator Solanum nigrum L. and non-hyperaccumulator Solanum lycopersicum L. Afr J Biotechnol 10(75):17180–17185
12. Gorelova SV, Garifzyanov AR (2012) Ascorbic acid as a factor in the anti-oxidant protection of woody plants at technogenic stress. Tula Ecol Bull 2012:177–183 (Russian)
13. Anjum NA, Ahmad I, Mohmood I et al (2012) Modulation of glutathione and its related enzymes in plants responses to toxic metal and metalloids. A review. Environ Exp Bot 75:307–324
14. Melancon MJ, Alscher R, Benson W et al (1992) Metabolic products as biomarkers. In: Huggett RJ, Kimerle RA, Mehrle Jr PM et al (eds) Biomarkers: biochemical, physiological and historical markers of anthropogenic stress. Lewis, Boca Raton, FL, pp 87–123
15. Van Gronsveld J, Clijsters H (1994) Toxic effects of metals. In: Farago M (ed) Plants and the chemical elements. Biochemistry, uptake, tolerance, and toxicity. VCH, New York, pp 149–178
16. Balakhnina T, Kosobryukhow A, Ivanov A, Kreslavskii V (2005) The effect of cadmium on CO_2 exchange, variable fluorescence of chlorophyll, and the level of antioxidant enzymes in pea leaves. Russ J Plant Physiol 52:15–20
17. Khan MA, Samiullah S, Singh S, Nazar R (2007) Activities of antioxidative enzymes, sulphur assimilation, photosynthetic activity and growth of wheat (Triticum aestivum) cultivars differing in yield potential under cadmium stress. J Agron Crop Sci 193:435–444
18. Gorelova SV, Menshikova EV, Charichonov AY (2013) Components of ascorbate-glutathione cycle of exotic species of wood in the conditions of anthropogenic stress. Factors plant resistance to extreme environmental conditions and technogenic environment. In: Materials Scientific Conference. SIFIBR SB RAS, Irkutsk, Russia, pp 60–63, 10–13 June 2013 (Russian)
19. Garifzyanov AR, Gorelova SV, Ivanischev VV, Muzafarov EN (2009) Comparative analysis of the activity of the components of the antioxidant system of woody plants in the conditions of anthropogenic stress News TSU. Natural sciences, vol 1. Tula State University Publishing House, Tula, pp 166–179 (Russian)
20. Gorelova SV, Charichonov AY (2013) Adaptation shrubs sanitary protection zone enterprises in the metallurgical industry to polymetallic pollution. In: Materials of III International scientific-practical conference "Plants and urbanization", Dnepropetrovsk, "Weasel". Dnepropetrovsk, pp 122–124, 19–20 March 2013 (Russian)
21. Bukharina IL, Povarnitskaya TM, Vedernikov KE (2007) Ecological and biological characteristics of woody plants in the urban environment: a monograph. FGOUVPO Izhevsk State Agricultural Academy, Izhevsk, 216 p (Russian)
22. Gorelova SV, Garifzyanov AR, Ivanishchev VV (2009) The response of photosynthetic pigments and some components of the antioxidant system of woody plants in the effect of aerosol emissions of metallurgical industry (the city of Tula). In: Problems of Modern Dendrology. Proceedings of the international scientific conference devoted to the 100th anniversary of Corresponding Member of the USSR Academy of PI Lapin. Association of Scientific Editions KMK, Moscow, pp 687–691, 30 June–2 July 2009 (Russian)
23. Gorelova SV, Volkova EM (2015) Physiological parameters of woody plants as the basis for bioindication and biomonitoring of the state natural and anthropogene ecosystems. News TSU. Natural Sciences, vol 3. Tula State University Publishing House, Tula, pp 271–283 (Russian)
24. Lichtenthaler HK, Rinderle U (1988) The role of chlorophyll fluorescence in the detection of stress conditions in plants. Critical Reviews in Analytical Chemistry. Vol. 19. Supplement 1. CRC Press, Inc. Pp. S29–S85
25. Krause GH, Weise E (1991) Chlorophyll fluorescence and photosynthesis: the basics. Annu Rev Plant Physiol Plant Mol Biol 42:313–349
26. Khudsar T, Mahmooduzzafar N, Iqbal M (2001) Cadmium-induced changes in leaf epidermis, photosynthetic rate and pigment concentrations in Cajanus cajan. Biol Plant 44(1):59–64
27. Soikkeli S, Karenlampi L (1984) Cellular and ultrastructure effects. In: Treshow M (ed) Air pollution and plant life. Wiley, Chichester, pp 159–174

28. Seregin IV, Kozhevnikova AD (2008) The role of root tissues and escape in the transport and accumulation of cadmium, lead, nickel and strontium. Plant Physiol 55(1):3–26. (Bibliogr.: pp 21–26, Russian)
29. Seregin IV (2009) Distribution of heavy metals in plants and their effect on growth. Abstract of dissertation for the degree of Doctor of Biological Sciences. M. “11format”, p 53 (Russian)
30. Wilkins DA (1957) A technique for measurement of lead tolerance in plants. Nature 180:37–38
31. Obroucheva NV, Bystrova EI, Ivanov VB, Antipova OV, Seregin IV (1998) Root growth responses to lead in young maize seedlings. Plant Soil 200:55–61
32. Obroucheva NV, Ivanov VB, Sobotik M, Bergmann H et al (2001) Lead effects on cereal roots in terms of cell growth, root architecture and metal accumulation. In: Gasparikova O et al (eds) Recent advances of plant root structure and function. Kluwer Academic, New York, pp 165–170. (Russian)
33. Gracheva VV, Seregin IV, Kozhevnikova AD, Ivanov VB (2008) Distribution of zinc in the tissues and organs of maize seedlings and growth inhibiting action. In: Proceedings of the International Conference “Physical–chemical bases of structural functional organization of plants”. Ekaterinburg, pp 150–151 (Russian)
34. Kulagin AA, Shagieva Y (2005) In: Rosenberg GS (ed) Woody plants and biological preservation of industrial pollutants. Nauka, Moscow, p 190 (Russian)
35. Gorelova SV, Garifzyanov AR (2008) Changing the anatomical and morphological leaf structure of woody plants in the affected area aerotechnogenic emissions of metallurgical enterprises (for example, the city of Tula). In: Nature of the Tula region and adjacent territories. Scientific Papers, vol 1. Grif and K, Tula, pp 172–179 (Russian)
36. Gorelova SV, Frontasyeva MV (2015) Biomonitoring of technogenic pollution in industrialized urban ecosystems using biogeochemical activity of woody plants. In: Book of abstracts BIOMAP7 “7th International Workshop on Biomonitoring of Atmospheric Pollution”. Instituto Superior Tecnoco Universidade de Lisboa, Lisbon, Portugal, p 83, 14–19 June 2015
37. Martin MH, Coughtrey PJ (1982) Biological monitoring of heavy metal pollution. Land and air. Applied Science, London. ISBN 0-85334-136–2
38. Markert B (1993) Instrumental analysis of plants. In: Markert B (ed) Plants as biomonitors. Indicators for heavy metals in the terrestrial environment. VCH, New York, pp 65–104
39. Markert B (ed) (1993) Plants as biomonitors. Indicators for heavy metals in the terrestrial environment. VCH Verlagsgesellschaft mbH, Weinheim, ISBN 3-527-30001-5 (Europe primarily determined by atmospheric deposition of these metals. J Soil Sediment 10:1572–1584)
40. Markert B, Oehlmann J, Roth M (2000) Biomonitoring of heavy metals: definitions, possibilities and limitations, In: Proceedings of an International Workshop on Biomonitoring of atmospheric pollution (with emphasis on trace elements)—BioMAP, Lisbon. IAEA, Vienna, Austria, p 129, 21–24 Sept 1997
41. Baker AJM (1981) Accumulators and excluders strategies in the response of plants to heavy metals. J Plant Nutr 3(1/4):643–654
42. Baker AJM, Walker PL (1990) Ecophysiology of metal uptake by tolerant plants. In: Shaw AJ (ed) Mercury metal tolerance in plants: evolutionary aspects. CRC, Boca Raton, FL, pp 156–177
43. Rühling A, Tyler G (1968) An ecological approach to the lead problem. Bot Notiser 12:321–342
44. Rühling A, Tyler G (1971) Regional differences in the heavy metal deposition over Scandinavia. J Appl Ecol 8:497–507
45. Rühling A, Tyler G (1973) Heavy metal deposition in Scandinavia. Water Air Soil Pollut 2:445–455
46. Ruhling A, Rasmussen L, Pilegaard K, Makinen A, Steinnes E (1987) Survey of atmospheric heavy metal deposition in the Nordic countries in 1985—monitored by moss analysis. NORD 21:44
47. Rühling A (1994) Atmospheric heavy metal deposition in Europe—estimations based on moss analysis. Nordic Council of Ministers, Copenhagen, p 9

48. Steinnes E (1980) Atmospheric deposition of heavy metals in Norway studied by moss analysis using neutron activation analysis and atomic absorption spectrometry. J Radioanal Chem 58:387–391
49. Steinnes E (1985) Use of moss in heavy metal deposition studies, EMEP/CCC-Report 3/85: 161–170.
50. Steinnes E, Andersson EM (1991) Atmospheric deposition of mercury in Norway: temporal and spatial trends. Water Air Soil Pollut 56:391–404
51. Steinnes E, Rambaek JP, Hanssen JE (1992) Large-scale multi-element survey of atmospheric deposition using naturally growing moss as biomonitor. Chemosphere 35:735–752
52. Steinnes E, Frontasyeva MV (1995) Epithermal neutronactivation analysis of mosses used to monitor heavy metaldeposition around an iron smelter complex. Analyst 120:1437–1440
53. Rühling Å, Steinnes E (1998). Atmospheric Heavy Metal deposition in Europe 1995–1996, NORD 1998:15. Nordic Council of Ministers, Copenhagen, Rounborgs grafiske hus, Holstebo, 1998:67
54. Berg T, Steinnes E (1997) Use of mosses (*Hylocomium splendens* and *Pleurozium schreberi*) as biomonitors of heavy metal deposition: from relative to absolute values. Environ Pollut 98:61–71
55. Schröder W, Holy M, Pesch R, Harmens H, Ilyin I, Steinnes E, Alber R, Aleksiayenak Y, Blum O, Coşkun M, Dam M, De Temmerman L, Frolova M, Frontasyeva M, González-Miqueo L, Grodzińska K, Jeran Z, Korzekwa S, Krmar M, Kubin E, Kvietkus K, Leblond S, Liiv S, Magnússon S, Maňkovská B, Piispanen J, Rühling Å, Santamaria J, Spiric Z, Suchara I, Thöni L, Urumov V, Yurukova L, Zechmeister HG (2010) Are cadmium, lead and mercury concentrations in mosses across Europe primarily determined by atmospheric deposition of these metals? J Soils Sediments 10:1572–1584
56. Harmens H, Norris D et al (2008) Spartial and temporal trends in heavy metal accumulation in mosses in Europe (1990–2005). RMG Design and Print Limited, Cromwell Court, UK, 51 p
57. Harmens H, Norris DA, Cooper DM, Mills G, Steinnes E et al (2011) Nitrogen concentrations in mosses indicate the spatial distribution of atmospheric nitrogen deposition in Europe. Environ Pollut 159:2852–2860
58. Harmens H, Norris D, Mills G, et al (2013) Heavy metals and nitrogen in mosses: spatial patterns in 2010/2011 and long-term temporal trends in Europe. ICP Vegetation Programme Coordination Center, Center for Ecology and Gidrology, Bangor, UK, p 63. ISBN: 978-1-906698-38-6. http://icpvegetation.ceh.ac.uk
59. Harmens H, Foan L, Simon V, Mills G (2013) Terrestrial mosses as biomonitors of atmospheric POPs pollution: a review. Environ Pollut 173:245–254
60. Harmens H, Mills G, Hayes F, Norris D. et al (2013) Air pollution and vegetation. ICP Vegetation annual report 2012/2013. ICP Vegetation Programme Coordination Centre, CEH Bangor, UK. ISBN: 978-1-906698-43-0. http://icpvegetation.ceh.ac.uk
61. UNECE ICP Vegetation (2008) Spartial and temporal trends in heavy metal accumulation in mosses in Europe (1990—2005). In: Harmens H, Norris D (eds) Participants of the moss survey. RMG Design and Print Limited, Cromwell Court, UK, p 51, July 2008
62. Barandovski L, Cekova M, Frontasyeva MV, Pavlov SS, Stafilov T, Steinnes E, Urumov V (2008) Atmospheric deposition of trace element pollutants in Macedonia studied by the moss biomonitoring technique. Environ Monit Assess 138:107–118
63. Saitanis J, Frontasyeva MV, Steinnes E, Palmer MV, Ostrovnaya TM, Gundorina SF (2013) Spatiotemporal distribution of airborne elements monitored with the moss bags technique in the Greater Thriasion Plain, Attica, Greece. Environ Monit Assess 185(1):955–964
64. Qarri P, Lazo PV, Stafilov T, Frontasyeva MV, Harmens H, Bekteshi L, Baceva K, Goryainova Z (2014) Multi-elements atmospheric deposition study in Albania. Environ Sci Pollut Res 21:2506–2518
65. Harmens H, Norris DA, Steinnes E, Kubin E, Piispanen J, Alber R, Aleksiayenak Y, Blum O, Coşkun M, Dam M, De Temmerman L, Fernández JA, Frolova M, Frontasyeva M, González-Miqueo L, Grodzińska K, Jeran Z, Korzekwa S, Krmar M, Kvietkus K, Leblond S, Liiv S,

Magnússon SH, Maňkovská B, Pesch R, Rühling Å, Santamaria JM, Schröder W, Spiric Z, Suchara I, Thöni L, Urumov V, Yurukova L, Zechmeister HG (2010) Mosses as biomonitors of atmospheric heavy metal deposition: spatial and temporal trends in Europe. Environ Pollut 158:3144–3156

66. Goltsova N, Vasina TV (1995) The use of bioindication for estimation of pollution in forest ecosystems of the Leningrad region. In*:* Bioindacators of environment health. Ecovision World Monograph Series. Academic, Amsterdam, pp 141–154
67. Vergel KN, Goryaynova ZI, Vihrova IV, Frontasyeva MV (2014) Method moss bio-monitors and GIS technologies in the assessment of air pollution by industrial enterprises of the Tikhvin district of Leningrad. Ecol Urban Area 2:92–101 (Russian)
68. Kharin VN, Fedorets NG, Shil'tsova GV, D'yakonov VV, Spektor EN (2001) Geographic trends in the accumulation of heavy metals in mosses and forest litters in Karelia. Russ J Ecol 32(2):138–141 (Translated from Ekologiya 2:155–158)
69. Ermakova EV, Frontasyeva MV, Steines E (2004) The study of the atmospheric deposition of heavy metals and other elements in the Tula region using the method of moss-biomonitors. Environ Chem 13:167–180 (Russian)
70. Ermakova EV, Frontasyeva MV, Steinnes E (2004) Air pollution studies in Central Russia (Tula region) using the moss biomonitoring technique, NAA and AAS. J Radioanal Nucl Chem 259:51–58
71. Ermakova EV, Frontasyeva MV, Pavlov SS, Povtoreiko EA, Steinnes E, Cheremisina YeN (2004) Air pollution studies in Central Russia (Tver and Yaroslavl Regions) using the moss biomonitoring technique and neutron activation analysis. Journal of Atmospheric Chemistry, vol 49. Kluwer Academic, New York, pp 549–561 (Printed in the Netherlands)
72. Vergel KN, Frontasyeva MV, Kamanina IZ, Pavlov SS (2009) Biomonitoring of heavy metals atmospheric deposition in north-east of Moskow region used by moss biomonitoring method. Ecol Urban Area 3:88–95 (Russian)
73. Dunaev AM, Latukhina KS, Abdalla AA, Rumyantsev IV, Nikiforov AY (2012) Study of heavy metal contents in soil, river water, snow, needles and mosses of Ivanovo Region. Neutron spectroscopy, Nuclear Structure, Related Topics. In: XIX International Seminar on Interactioon of Neutrons with Nuclei. JINR, Dubna, pp 320–326, 25–28 May 2011
74. Dunaev AM, Rumyantsev IV, Frontasyeva MV, Grinevich VI (2014) Atmospheric air contamination assessment in Ivanovo Region by means of combined analysis of snow and mosses. Fundamental interactions & neutrons, nuclear structure, ultracold neutrons, related topics. In: XXI International Seminar on Interactioon of Neutrons with Nuclei, Alushta, Ukraine. JINR, Dubna, pp 282–286, 20–25 May 2013
75. Smirnov LI, Frontasyeva MV, Steines E (2004) Multivariate statistical analysis of heavy metals and radionuclides concentration in the mosses and soil of South Ural. Atomic Energy 97(1):68–74
76. Frontasyeva MV, Smirnov LI, Steines E, Lyapunov SM (2004) Cherchintsev VD. Heavy metal atmospheric deposition study in the South Ural mountains. J Radioanal Nucl Chem 259:19-26.
77. Pankratova Yu S, Frontasyeva MV, Berdnikov AA, Pavlov SS (2007) Air pollution studies in the Republic of Udmurtia, Russian Federation, using moss biomonitoring and INAA. In: Granja C, Leroy C, Stekl I (eds) Proceedings of Summer School "Nuclear Physics Methods and Accelerators in Biology and Medicine—2007", AIP Conference Proceedings, vol 958. American Institute of Physics, New York, pp 236–237
78. Koroleva YV, Krasnov EV (2002) Air pollution in Kaliningrad Region. The use and protection of natural resources in Russia 5–6:144–146 (in Russian)
79. Koroleva YuV (2006) Use of mosses (Hylocomium splendens, Pleurozium schreberi) to evaluate the absolute values of the atmospheric deposition of TM in the Kaliningrad region. Bulletin of the Kant Russian State University. Series of natural science, vol 7. Immanuel Kant, Kaliningrad, pp 29–34
80. Koroleva YV (2010) Bioindication atmospheric deposition of heavy metals in the Kaliningrad region. Kant Russian State University. Bulletin of the Russian State Kant University. Series of natural science, vol 7. Immanuel Kant, Kaliningrad, pp 39–44

81. Koroleva YV, Puhlova IA (2012) New data on the bioconcentration of heavy metals in the Baltic region. Bulletin of the Russian State Kant University. Series of natural science, vol 7. Immanuel Kant, Kaliningrad, pp 99–107
82. Koroleva YV, Vakhraynova O, Styogantsev V, Melnikova I (2014) Accumulation of heavy metals in the moss *Pleurozium schreberi* in the west of Russia (Kaliningrad region). In: 27th Task Force Meeting and Ozone Workshop. ICP Vegetation, Programme and Abstracts, Paris, France, p 64, 28–30 Jan 2014
83. ICP Vegetation (2016) 29th Task Force Meeting. Dubna, Russian Federation. Programme and Abstracts, JINR, p 82, 29th Feb–4th Mar 2016
84. Wegener JWM, Van Schaik MJM, Aiking H (1992) Active biomonitoring of polycyclic aromatic hydrocarbons by means of mosses. Environ Pollut 76:15–18
85. Thomas W, Schunke E (1984) Polyaromatic hydrocarbons, chlorinated hydrocarbons and trace metals in moss samples from Iceland. Lindbergia 10:27–32
86. ICP Vegetation (2013) Ozone pollution: impacts on ecosystem services and biodiversity. Report prepared by the ICP Vegetation, April 2013. In: Mills G, Wagg S, Harmens H (eds) Center for Ecology and Gidrology, Bangor, UK, p 104
87. ICP Vegetation (2014) 27th Task Force Meeting and Ozone Workshop. Programme and Abstracts, Paris, France, p 77, 28–30 January 2014
88. Alfani A, Bartoli G, Caserta P, Andolfi G (1995) Amount and elemental composition of dry deposition to leaf surface of *Quercus ilex* in the urban area of Naples. Agr Mediterranea Spec Vol 15:194–199
89. Djingova R, Wagner G, Peshev D (1995) Heavy metal distribution in Bulgaria using *Populus nigra "Italica"* as a biomonitor. Sci Total Environ 172:151–158
90. Lukina NV, Nikonov VV (1996) Biogeochemical cycles in the North woods in terms of environmental contamination. Part 2. Publishing House of the Kola Science Centre of Russian Academy of Sciences, Apatity, p 192 (Russian)
91. Mankovska B, Popierová D, Florek M, Frontasyeva MV, Ermakova E, Antoni J (2003) Elemental composition of lime wood response to atmospheric deposition. Ekologia Bratislava 22(1):152–156
92. Tomašević M, Rajšić S, Đorđević D, Tasić M, Krstić J, Novaković V (2004) Heavy metals accumulation in tree leaves from urban areas. Environ Chem Lett 2:151–154
93. Tomasević M, Vukmirović Z, Rajšić S, Tasić M, Stevanović B (2005) Characterization of trace metal particles deposited on some decidous tree leave in an urban area. Chemosphere 61:753–760
94. Chernenkova TV (2002) The response of forest vegetation on industrial pollution. Nauka, Moscow, p 190 (Russian)
95. Ufimtseva MD, Terekhina NV (2005) Phytoindication ecological state urbogeosistem. Science, St. Petersburg, p 339 (Russian)
96. Gorelova SV, Garifzyanov AR, Lyapunov SM, Gorbunov AV, Okina OI, Frontasyeva MV (2010) The assessment of trees using possibility for bioindication and biomonitoring of metallurgical industry emissions. In: Heavy metals and radionuclides in the environment. Materials of VI International Scientific-Practical Conference. Semipalatinsk, pp 299–306, 4–10 Feb 2010 (Russian)
97. Gorelova SV, Garifzyanov AR, Frontasyeva MV, Lyapunov SM, Gorbunov AV, Okina OI (2010) Physiological stability as a factor for selection of woody plants for phytoremediation and biomonitoring. In: International Conference on Environmental Pollution and CleanBio/Phytoremediation, Pisa, Italy, p 21, 16–19 June 2010
98. Gorelova SV, Frontasyeva MV, Yurukova L, Coskun M, Pantelica A, Saitanis CJ, Tomasevic M, Anicic M (2011) Revitalization of urban ecosystems through vascular plants: preliminary results from the BSEC-PDF project. Agrochimica 55(2):65–84
99. Gorelova SV, Frontasyeva MV, Lyapunov SM, Gorbunov AV, Okina OI (2011) Woody plants in passive biomonitoring and bioremediation of urban ecosystems. Aspect of heavy metal accumulation. Neutron spectroscopy, nuclear structure, related topics. In: Abstract of the XIX International Seminar on Interaction of Neutrons with Nuclei, Dubna. JINR, Dubna, p 29, 25–28 May 2011

100. Gorelova SV, Frontasyeva MV (2012) Concentration factor (Cf) of the essential elements in woody plants. Fundamental interactions & neutrons, nuclear structure, ultracold neutrons, related topics. In: Abstract of the XX International Seminar on Interaction of Neutrons with Nuclei, Alushta, Ukraine. JINR, Dubna, p 44, 21–26 May 2012
101. Gorelova SV, Frontasyeva MV, Gorbunov AV, Lyapunov SM, Okina OI (2014) Bioindication and monitoring of atmospheric deposition using trees and shrubs. Materials of 27th Task Force Meeting of the UNECE ICP Vegetation. Paris, France, p 63, 28–29 Jan 2014
102. Gorelova SV, Frontasyeva MV, Gorbunov AV, Lyapunov SM, Okina OI (2014) Biogeochemical activity of some coniferous exotic woody species in urban ecosystems. Fundamental interactions & neutrons, nuclear structure, ultracold neutrons, related topics. In: Abstract of the XXII International Seminar on Interaction of Neutrons with Nuclei, Dubna. JINR, Dubna, 25–30 May 2014
103. Gorelova SV, Volkova ME, Frontasyeva MV (2015) Bioindication and biomonitoring of anthropogenic pollution of ecosystems using a biogeochemical characteristics of the leaves of woody plants. News TSU. Natural sciences, vol 4. Tula State University Publishing House, Tula, pp 232–248 (Russian)
104. Gorelova SV, Frontasyeva MV, Lyapunov SM, Gorbunov AV, Mochalova EG, Okina OI (2015) Biogeochemical activity of exotic *Gimnospermae* species in the industrialized urban ecosystems. Journal of I, vol 1. Kant Baltic Federal University, pp 92–106 (Russian)
105. Gorelova SV, Kozlov SA, Tolkunova EY, Lyapunov SM, Gorbunov AV, Okina OI, Frontasyeva MV (2015) The study of atmospheric air in industrial areas of Tula city. Biology—Science of XXI Century. In: 19th International Conference Pushchino School of Young Scientists, Pushchino, Abstracts. Pushchino, p 418, 20–24 April 2015
106. Kuznetsov EA et al (2012) Applied ecobiotechnology: a tutorial, 2nd edn, vol 2. Binom. Knowledge Laboratory, Moscow, p 485, ISBN 978-5-9963-1052-4 (T. 2), ISBN 978-5-9963-0777-7 (in Russian) http://znanium.com/catalog.php?bookinfo=368953
107. Gorelova SV (2015) Use of exotic Gimnospermae species for biomonitoring of environment in the urban ecosystems. Ecological problems of industrial cities. In: Scientific Papers of the 7th Russian Scientific-Practical Conference with International Participation. Saratov, vol 2. SGTU, pp 48–52 (Russian)
108. Prasad MN (2003) Practical use of plants for remediation of ecosystems contaminated with metals. Physiol Plant 50(5):764–780 (Russian)
109. Baker AJM, Brooks RR (1989) Terrestrial higher plants which hyperaccumulate metallic elements—a review of their distribution, ecology and phytochemistry. Biorecovery 1:81–126
110. Brooks RR (1998) Plants that hyperaccumulate heavy metals. CAB International, Wallingford, 384 p
111. Prasad NNV (2007) Sunflower (*Helianthus Anuus* L.)—a potential crop for environmental industry. Helia 30(46):167–174
112. Komarek M, Tlustos P, Szakova J, Richner W, Brodbeck M, Sennhauser M (2007) The use of maize and poplar in chelant-enchanced phytoextraction of lead from contaminated soils. Chemosphere 67(4):640–651
113. Pulford ID, Watson C (2003) Phytoremediation of heavy metal-contaminated land by trees—a review. Environ Int 29:529
114. Kolbas A, Mench M, Herzig R, Nehnevajova E, Bes SM (2011) Copper phytoextraction tandem with oilseed production using commercial cultivars and mutant lines of sunflower. Int J Phytoremediat 13(1):149–165
115. Prasad MNV, Nakbanpote W, Phadermrod C, Rose D, Suthari S (2016). Mulberry and vetiver for phytostabilization of mine overburden: cogeneration of economic products. In: Prasad MNV (ed) Bioremediation and bioeconomy. Elsevier, Amsterdam, pp 295–328. ISBN 978-0-12-802830-8
116. Sathya A, Kanaganahalli V, Srinivas Rao P, Gopalakrishnan S (2016). Cultivation of sweet sorgum on heavy metal-contaminated soils by phytoremediation approach for production of bioethanol. In: Prasad MNV (ed) Bioremediation and bioeconomy. Elsevier, Amsterdam, pp 272–289. ISBN 978-0-12-802830-8

117. Kumar PBAN, Dushenkov V, Motto H, Raskin I (1995) Phytoextraction: the use of the plants to remove heavy metals from soils. Environ Sci Technol 29:1232–1238
118. Abou-Shanab RA, Chanem K, Chanem N, Al-Kolaibe A (2008) The role of bacteria on heavy metal extraction and uptake by plants growing on multi-metal-contaminated soils. World J Microbiol Biotechnol 24:254–262
119. Rajkumar M, Sandhya S, Prasad MNV, Freitas H (2010) Perspectives of plant-associated microbes in heavy metal phytoremediation. Biotechnology 30:1562–1574
120. Vedernikov KE, Pashkov EV, Alekseenko AS (2012) Features studying mycorhiza of woody plants in the conditions of urbano areas. In: Materials All-Russian Scientific-Practical Conference, vol 1. VPO Izhevsk State Agricultural Academy, Izhevsk, pp 259–261 (Russian)
121. Carlot M, Ciacomini A, Casella S (2002) Aspects of plant-microbe interaction in heavy metal polluted soil. Acta Biotechnol 22:13–20
122. Ganesan V (2008) Phytoremediation of cadmium soil using a cadmium-resistant plant growth-promoting rhizopseudomonad. Curr Microbiol 56:403–407
123. Siegel FR (2002) Environmental geochemistry of potentially toxic metals. Springer, Heidelberg, 218 p
124. Salt DE, Blaylock M, Kumar NP, Dushenkov V, Ensley BD, Chet I, Raskin I (1995) Phytoremediation: a novel strategy for the removal of toxic metals from the environment using plants. Biotechnology 13(5):468–474
125. Dushenkov VP, Kumar PBAN, Motto H, Raskin I (1995) Rhizofiltration: the use of plants to remove heavy metals from aqueous streams. Environ Sci Technol 29(5):1239–1245
126. Lee M, Yang M (2010) Rhizofiltration using sunflower (*Helianthus annuus* L.) and bean (*Phaseolus vulgaris* L. var. vulgaris) to remediate uranium contaminated groundwater. J Hazard Mater 173:589-596
127. Brooks RR, Chambers MF, Nicks W, Robinson BH (1998) Phytomining. Trends Plant Sci 3:359–362
128. Verma VK, Singh YP, Rai JNP (2007) Biogas production from plant biomass used for phytoremediation of industrial wastes. Bioresour Technol 98:1664–1669
129. Kathi S. (2016) Bioenergy from phytoremediated phytomass of aquatic plants via gasification. In: Prasad MNV (ed.) Bioremediation and bioeconomy. Elsevier, Amsterdam, pp 111–128. ISBN 978-0-12-802830-8
130. Wenzel WW, Lombi E, Adriano D (1999) Biogeochemical processes in the rhizosphere: role in phytoremediation of metal-polluted soils. In: Prasad N, Hagemeyer J (eds) Heavy metal stress in plants—from molecules to ecosystems. Springer, Heidelberg, pp 273–303
131. Glass DJ (1999) U.S. and International markets for phytoremediation, 1999–2000. D. Glass Associates, Needham, MA, p 270
132. Palmer CE, Warwick S, Keller W (2001) *Brassicaceae (Cruciferae)* family, plant, biotechnology and phytoremediation. Int J Phytoremediat 3:245–287
133. Prasad MNV (2004) Phytoremediation of metals and radionuclides in environment: the case for natural hyperaccumulators, metal transporters, soil-amending chelators and transgenic plants. In: Prasad MNV (ed) Heavy metal stress in plants: from biomolecules to ecosystems, 2nd edn. Springer, Berlin, pp 345–391
134. Prasad MNV (ed) (2016) Bioremediation and bioeconomy. Elsevier, Amsterdam, p 698. ISBN 978-0-12-802830-8
135. Prasad MNV, Sadzhavana KS, Naidu P (eds) (2009) Trace elements in the environment: biogeochemistry, biotechnology and bioremediation. (trans: from English Bashmakov DI, Lukatkin AS). FIZMATLIT, Moskow, 816 p. (Russian)
136. Favas PJC, Pratas J, Chafurvedi R, Paul MC, Prasad MNV (2016) Tree crops on Abandoned mines for environmental remediation and industrial feedstock. In: Prasad MNV (ed) Bioremediation and bioeconomy. Elsevier, Amsterdam, pp 219–251. ISBN 978-0-12-802830-8
137. Pulford ID, Dickinson NM 2009 The use of trees in phytoremediation technologies. In: Prasad MNV, Sadzhavana KS, Naidu P (eds) Trace elements in the environment: biogeo-

chemistry, biotechnology and bioremediation (translation from English Bashmakov DI, Lukatkin AS). FIZMATLIT, Moscow, pp 440–463

138. Coates W (2005) Tree species selection for a mine tailings bioremediation project in Peru. Biomass Bioenergy 28:418–423
139. Sheoran V, Sheoran AS, Poonia P (2009) Phytomining: a review. Miner Eng 22:1007–1019
140. Bridgewater AV (2012) Upgrading biomass fast pyrolysis liquids. Environ Prog Sustain Energy 31(2):261–268
141. Evangelou MNH, Papaglou EG, Robinson BH, Schulin R (2015) Phytomanagement: phytoremediation and the production of biomass for economic revenue on contaminated land. In: Ansari AA et al (eds) Phytoremediation: management of environmental contaminants, vol 1. Springer, New York, pp 115–132
142. Hooper DU, Vitousek PM (1997) The effects of plant composition and diversity on ecosystem processes. Science 277:1302–1305
143. Freitas H, Prasad MNV, Pratas J (2004) Plant community tolerant to trace elements growing on the degraded soils of Sao Domingos mine in the south east of Portugal: environmental implications. Environ Int 30:65–72
144. Chibrik TS, Lukina NV, Filimonova FI, Glazyrina MA, Rakov EA, Maleva MG, Prasad MNV (2016) Biological recultivation of mine industry deserts: facilitating the formation of phytocoenosis in the middle Ural region, Russia. In: Prasad MNV (ed) Bioremediation and bioeconomy. Elsevier, Amsterdam, pp 389–418. ISBN 978-0-12-802830-8
145. Gorelova SV, Kozlov SA, Tolkacheva EY, Gorbunov AV, Lyapunov SM (2014) Ecological state of soil of Tula city. In: Biology—Science of XXI Century: the 18th International Conference Pushchino School of Young Scientists, Pushchino. Abstracts. Pushchino, pp 410–411, 21–25 Apr 2014 (Russian)
146. Methodological guidelines for determination of heavy metals in soils and agricultural crop production (1992) CINAO, Moscow, 61 p (in Russian).
147. Frontasyeva MV, Pavlov SS (1999) REGATA experimental setup for air pollution studies. Problems of modern physics. In: Sisakyan AN, Trubetskov VI (eds). JINR, Dubna, pp 152–158
148. Markert B (1992) Establishing of “Reference Plant” for inorganic characterization of different plant species by chemical fingerprinting. Water Air Soil Pollut 64:533–538
149. Kabata-Pendias A, Pendias H (2001) Trace elements in soil and plants, 3rd edn. CRC, Boca Raton, FL, p 432
150. Bityutsky NP (2011) Trace elements of higher plants. Publishing House of Saint-Petersburg University, Saint-Petersburg, 368 p
151. Riddell-Black D (1994) Heavy metal uptake by fast growing willow species. In: Aronsson P, Perttu K (eds) Willow vegetation filters for municipal wastwaters and sludges: a biological purification system. Swedish University of Agricultural Sciences, Uppsala, p 145
152. Greger M, Landberg T (1999) Use of willows in phytoextraction. Int J Phytoremediat 1:115
153. Watson C (2002) The phytoremediation potential in *Salix:* studies of the interaction of heavy metals and willows. Ph.D. Thesis. University of Glasgow
154. Hammer D, Kayser A, Keller C (2003) Phytoextraction of Cd and Zn with *Salix wiminalis* in field trials. Soil Use Manage 19:187
155. Laureysens I, Deeraedt W, Indeherberge T, Ceulemans R (2003) Population dynamics in 6-year old coppice culture of poplar. I. Clonal differences in stool mortality, shoot dynamics and shoot diameter distribution in relation to biomass production. Biomass Bioenergy 24:81
156. Laureysens I, Blust R, De Temmerman L, Lemmens L, Ceulemans R (2004) Clonal variation in heavy metal accumulation and biomass production in poplar coppice culture. I. Seasonal variation in leaf, wood and bark concentrations. Environ Pollut 131:485–494
157. Laureysens I, Bogaert J, Blust R, Ceulemans R (2004) Biomass production of 17 poplar clones in a short-rotation coppice culture on a waste disposal site and its relation to soil characteristics. Forest Ecol Manag 187:295
158. Laureysens I, De Temmerman L, Hastir T, Van Gysel M, Ceulemans R (2005) Clonal variation in heavy metal accumulation and biomass production in poplar coppice culture. II. Vertical distribution and phytoaxtraction potencial. Environ Pollut 133:541

159. Ekvall L, Greger M (2003) Effects of environmental biomass-producing factors on Cd uptake in two Swedish ecotypes of Pinus sylvestris. Environ Pollut 121:401
160. Berry C (1985) Growth and heavy metal accumulation in *Pine* seedlings grown with sewage sludge. J Environ Qual 14:415
161. Saqib ANS, Waseem A, Khan AF, Mahmood Q et al (2013) Arsenic bioremediation by low cost materials derived from blue pine (*Pinus wallichiana*) and walnut (*Juglans regia*). Ecol Eng 51:88–94
162. Turner AP, Dickinson NM (1993) Survival of *Acer pseudoplatanus* L. (sycamore) in tissue culture. New Phytol 123:523
163. Turner AP, Dickinson NM (1993) Cooper tolerance of *Acer pseudoplatanus* L. (sycamore) in tissue culture. New Phytol 123:509
164. Ehlin PO (1982) Seasonal variations in metal contents of birch. Geologiska Forningens Stockholm Fohandkingar 104:63
165. Borgegard SO, Rydin H (1989) Biomass, root penetration and heavy metal uptake in birch in the soil cover over copper tailings. J Appl Ecol 26:585
166. Eltop L, Brown G, Joachim O, Brinkmann K (1991) Lead tolerance of Betula and salix in the mining area of Mechernich, Germany. Plant Soil 131:275
167. Ultriainen MA, Karenlampi LV, Karenlampi SO, Schat H (1997) Differential tolerance to copper and zinc of morfopropagated birches tested in hydroponics. New Phytol 137:543
168. Kopponen P, Utriainen M, Lukkari K, Suntioinen S, Karenlampi L, Karenlampi S (2001) Clonal difference in cooper and zinc tolerance of birch in metal-supplemented soils. Environ Pollut 112:89
169. Prasad MNV, Freitas H (2003) Removal of toxic metals from solution by leaf, stem and root phytomass of *Quercus ilex* (hobly oak). Environ Pollut 110:277–283
170. Wisniewsi L, Dickinson NM (2003) Toxity of copper to *Quercus robur* (English oak) seedlings from a cooper-rich soil. Environ Exp Bot 50:99
171. Pyatt FV (2001) Cooper and lead accumulation by Acacia retinoides and Eucaliptus torquata in sites contaminated as a consequence of extencive ancient mining activities in Cyprus. Ecotoxicol Environ Saf 50:60
172. Chockalingam E, Subramanian S (2009) Utility of *Eucalyptus tereticornis* bark and Desulfotomaculum nitrificans for the remediation of acid mine drainage. Bioresour Technol 100:615–621
173. Hovmand MF, Nielsen SP, Johnsen I (2009) Root uptake of lead by Norway spruce grown on ^{210}Pb spiked soils. Environ Pollut 157:404–409
174. Monaci F, Bargagli R (1995) Biomonitors as a tool to evaluate trace element deposition in complex urban environments. In: Proceedings of the 10th Word Clear Air Congress held in 1995, Helsinki, pp 238–241
175. Gorelova SV, Frontasyeva MV, Lyapunov SM, Gorbunov AV, Okina OI (2014) Perspectives of woody plant implementation in phytoremediation of toxic elements in industrial cities. In: Book of abstracts “11th International Phytotechnology Conference” Heraklion, Crete, Greece, p 333, 30 Sept–3 Oct 2014
176. Paribok TA (1975) On accumulation of rare and scattered elements by plants of far-north. Physiological role of trace elements in plants. Edt. Shkol’nik M.Ya. Moscow, pp 184–194 (Russian)
177. Kemp K (2002) Trends and sources for heavy metals in urban atmosphere. Nucl Instrum Methods Phys Res, Sect A 189:227–232
178. Burkhardt J (2010) Hygroscopic particles on leaves: nutrient or desiccants? Ecol Monogr 80(3):369–399