Soil toxicity reduction by phytoindicators

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Abstract. The grass mixtures use in phytoremediation is poorly studied, so we have proposed some options for new plant species that can grow on polluted land and accumulate pollutants with aboveground biomass. In heavy soil contamination case with heavy metals, we suggest using annual plants with large biomass in the recultivation first year, which can remove up to 50% of the pollutant from the biological cycle in the first year. Studying plants on toxic soils, we determined their accumulating capacity and used them as phytomeliorants. In several experiments, indicator plants were used: amaranth, clover, alfalfa, elm, stevia, ragweed, winter camelina mixed with an annual type of clover, and others, which, with the maximum biomass accumulation, were plowed into the soil in a mixture with zeolite-containing clays of local importance. The experiments result showed that with the indicator plants help it is possible not only to improve soil fertility, but also to significantly reduce the heavy metals and oil products amount. Organic waste from agricultural production was also of great importance in reducing soil toxicity: corn cobs, sunflower baskets, and leaf litter embedded in the soil with biological products. To activate the plowed organic mass, para-aminobenzoic acid (PABA) was used. The experiments result showed a significant decrease in toxicants in the soil when using organic waste and plowing plants as a green fertilizer in a mixture with zeolite-containing clays and biological products.

1. Introduction

To preserve close to natural vegetation, as well as for plant growing and forestry purposes in conditions of anthropogenic pollution, particular importance is attached to the question of how much existing plants are already resistant to heavy metals. There are several ways to check this, for example, the root growth comparative measurement and the comparative protoplasmic method [1, 2, 3].

Impacts on soil are not uniform depending on the pollution source in densely populated areas, which usually occupy convenient and advantageous locations. For this reason, the soils’ purification (restoration) from pollutants excess masses is a very urgent task. Its practical solution is still at the development stage. The possible ways one to solve this problem can be phytoremediation - cleaning the soil from pollution by cultivating plants that actively absorb pollutants. Phytoremediation is a highly efficient technology for removing an organic substances number. Plants can be used for cleaning solid, liquid and airy substrates [4, 5, 6]. Contaminated soils and sedimentary rocks phytoremediation is already being used to clean up military landfills (from metals, organic pollutants), agricultural land (pesticides, metals, selenium), industrial zones (organics, metals, arsenic), and wood processing sites.
Phytoremediation can be applied to urban wastewater, agricultural and industrial wastewater, groundwater [7, 8]. Such plants can serve corn, ginger, mustard, lettuce, oats, barley, peas, beans, perennial herbs and others. Grass mixtures should be selected for these regions specific climatic conditions, taking into account the individual crops ability to accumulate pollutants in the aboveground biomass [9, 10]. They should also be undemanding to soil fertility (mesotrophic or oligotrophic) and growing capacity in severe pollution conditions. The main criterion for the proposed mixtures' classification was the land disturbance or contamination method [11, 12].

2. Research methodology

Our research was aimed at rehabilitating contaminated soils using plants with sorption capacity. These are amaranth plants, legumes (their crop residues), legumes, ragweed, stevia, corn cobs, sunflower baskets, oilseeds (camelina, gvisocia, krambe and others).

Together with plants, we introduce zeolite-containing clays of local origin (mountains and foothills of the North Caucasus), as well as biofertilizers, which ensure the disturbed lands' restoration, to recommend soils. The experiments were carried out at the Gorsk State Agrarian University experimental sites, the Russian Academy of Sciences Vladikavkaz Scientific Center North Caucasian Research Institute of Mining and Piedmont Agriculture, and the North Ossetian State University named after K.L. Khetagurov.

In another experiment, to reduce the soils' radioactivity, the leguminous herbs' seeds were enveloped with a crushed sunflower heads and corn stalks mixture, Alanite clay and molasses in a ratio of 1: 1: 10: 1. On the mown plot at the growing season end, woody crops fallen leaves layer was placed in a mixture with alanite at a dose of 2–2.05 t/ha [9, 10]. Small-seeded perennial legumes were sown with the creeping clover (Trifolium repens L.) advantage - 8 kg/ha, alfalfa (Medicago Sativa L.) - 6 kg/ha Oriental goat's rue (Galega orientalis Lam) - 6 kg/ha. The legumes total mixture was 20 kg/ha. Taking into account the creeping clover peculiarities to spread root suckers over the territory, covering the area already in the life first year, this grass type seeding rate was increased as a component with a greater assimilation surface for sorbing heavy metals.

Before sowing, the leguminous herbs' seeds were enveloped with crushed stalks and sunflower heads mixture in an equal proportion, 5 kg/ha of each component. To them were added 50 kg/ha of Alanite - zeolite-containing clay and as a binder - molasses - starch production waste 5 kg/ha.

The studied areas' soils are mainly represented by medium-thick heavy loamy leached chernozem, underlain by pebbles with a coarse sand large amount in the upper horizons (8-14%). This soil type, as a rule, has a high water-holding capacity with a hummus and nutrients sufficient content and has good physical properties. In some places, pebbles come to the surface. The leached chernozems soil solution reaction ranges from slightly acidic to close to neutral (pH of salt extract 5.48-6.92).

Zeolite-containing clay Alanite - local origin with the content of macro - and microelements was widely used for research. Silicon contained in clay (about 50%) has a high sorption capacity, absorbing toxic substances. The raw materials natural sources contain clay particles within 30-40%, coarse-grained inclusions within 2-15%. Alanite has a slightly alkaline reaction on soils with an acidic environment. Clay Alanite, having a high calcium content and alkaline reaction (pH - 9.3), reduces the soil acidity in the contaminated area.

Corn cobs - waste of starch - syrup production a grain corn 1 ton contains 200 kg of rods) have high solubility, sorption capacity, the medium (pH -6.9-7.1) neutral reaction, no resins, wax, heavy metals complete absence and a trace elements complex. All these indicators characterize corn cobs as ideal organic carriers.

Corn cobs contain 41.7% cellulose; 37.2% hemicellulose; 8% lignin; 0.08% fat; 1.75% protein; NFES -61.7%. When crushing, the protein content in the stubs increases to 4.34-1%; NFES -65.1% [8, 9].
Calcium contained in alanite provides a decrease in the acidity of vinasse, neutralizing the applied alanite substrate. The humid environment of vinasse and cabbage stalks reduces the dust particles amount in contaminated areas [9, 10].

Molasses - sugar production waste - contains 20-25% water, mainly amides; 58-60% carbohydrates, mainly sugar and 7-10% ash. In this object, as a binding mixture of corn stalks, sunflower baskets, alanite is used in the amount of 5 kg/ha.

On a site charged with heavy metals (lead and cadmium, iron, zinc, copper, nickel), with an area of 1 hectare in the mining industry (the Unal RSO-A village), seeds were prepared for sowing at the rate of creeping clover - 8 kg /ha, alfalfa changeable - 6 kg/ha Oriental goat's rue - 6 kg/ha. Crushed corn stalks and sunflower baskets at 5 kg/ha each. To them were added 50 kg/ha of alanite and 5 kg/ha of molasses. All ingredients were mixed and the seeds were coated in a pelletizer, then sown with a conventional grain seeder.

In the budding beginning phase, the green mass in the sowing year was mowed and disposed of in specially prepared trenches for burial.

At the growing season end - in autumn, the contaminated area was covered with a leaf litter layer from tree crops collected from forest park areas in the 1-2 tons amount per hectare, which were mixed with Alanite 2-2.5 tons.

On oil-contaminated soils, a remediation effective method is the para-aminobenzoic acid (PABA) use mixed with a stevia plant 0.2% aqueous solution, and an amaranth culture was sown 2-3 weeks after watering.

A working PABA liquid was prepared at the rate of 100 g of powder per 10 litres of water, with a temperature of 80°C. Stevia leaves in an amount of 200 g were dipped into hot water. In the fresh leaves absence, stevia preparations were used, available in any pharmacy. In this case, 20 ml of stevia solution was used per 10 litres of liquid. A biological product Nikfan solution was prepared at the same time at the rate of 1 part per 100 parts of water, that is, 100 g of the biological product per 10 litres of water. After cooling the PABA solution to 20-25°C two solutions were mixed, the total of which was 20 litres. This solution amount was used to irrigate an oil-contaminated area of 10 m².

3. Research results

The site enriched with organic substances significantly reduces toxicity and restores the fertile layer suitable for the cultivation of crops. Moreover, due to the addition of corn stalks to the alanite substrate, the site aeration and its filtration are improved. The experiment results are summarized in table 1.

| Experiment variants | Pb  | Zn   | Cu   | Cd  | Fe   | P$_h$ |
|---------------------|-----|------|------|-----|------|------|
| Control (no improvement) | 12.9 | 136.0 | 78.0 | 0.64 | 28.0 | 4.2  |
| Alanite layer application | 8.6 | 8.0  | 39.0 | 0.52 | 22.0 | 5.4  |
| Corn stalks | 5.6 | 39.0 | 21.0 | 0.34 | 16.0 | 5.9  |
| Ridge formation + alanite layer | 2.1 | 14.0 | 13.6 | 0.22 | 9.6  | 6.2  |
| Alanite clay + corn stalks + perennial grasses sowing | 0.2 | 8.0  | 9.2  | 0.01 | 4.5  | 6.8  |

Consequently, natural zeolite-containing clays mixed with corn stalks can rehabilitate lands disturbed by mining, reduce the heavy metals and soil acidity (Ph) amount without special costs, while simultaneously utilizing crop products.

Ragweed – an American origin quarantine weed [01] – has a wide distribution in Russia (the far East, Siberia, Central and the country European part southern regions, etc.). The main area occupied by ragweed is in the North Caucasus, Rostov and Volgograd regions and Primorsky Krai. Along the
railways and highways, ragweed is introduced to the northern regions of Russia (Komi, Karelia, Murmansk Region). The plant is unpretentious, it is found in large quantities and almost everywhere, in various environmental conditions, without avoiding technogenically polluted territories. The growing season is long and is 150-170 days. The flowering phase lasts more than 60 days, which makes it possible to collect the aboveground mass during the heavy metals maximum accumulation period. According to their content, in this phase, it is easy to identify plants with high sorption capacity.

The different plants' sorption capacities comparison in the same developmental phases, but different environmental conditions, makes it possible to identify species and crops with maximum bioindication capabilities. To quantitatively determine the ragweed ability to accumulate heavy metals in the aboveground mass, in comparison with other crops with similar sorption properties (clover, alfalfa, sainfoin), experiments were carried out on a metallurgical plant territory near the highway and in agricultural land. It was found from the experiments that ragweed has the maximum accumulating ability to accumulate heavy metals. Taking into account the peculiarity of vascular plants to concentrate heavy metals at the growing season beginning the growing season in a minimum amount, with a gradual increase in their content towards the flowering phase.

To reduce soil toxicity (with the herbicides' introduction), amaranth plants were sown in the corn aisles, which were fed with the biological product Nikfan during the growing season, after which, by processing the aisles, the amaranth green mass was embedded in the soil (table 2).

Table 2. Soil remediation with amaranth mixed with Nikfan biological product.

| Experiment variants                                                                 | Contents nitrogen's | Heavy metals' content |
|-------------------------------------------------------------------------------------|---------------------|-----------------------|
| Control (without herbicides and biological product)                                 | 172.0               | 15.8                  |
| Herbicide application                                                               | 121.0               | 22.6                  |
| Crops treatment with herbicides and amaranth overseeding in the aisle               | 180.0               | 13.2                  |
| Corn crops processing and the biological product Nikfan introduction               | 198.0               | 8.2                   |
| Sowing amaranth in aisles + introducing a biological product + amaranth green mass incorporating as green manure | 224                 | 6.4                   |
| Maximum permissible concentration (MPC)                                             | 32.0                | 6.8                   |

From the above data, it follows that in the proposed version, due to the amaranth plants (Amaranthus caudatus) incorporation into the soil with a biological product, the biological nitrogen amount increases, the heavy metals content decreases to maximum permissible concentrations and below.

The variegated elm, in contrast to the well-known vetch and other legumes sown on contaminated soils, has a root-sprouting system, and propagates both by seeds and vegetatively (by root-sprouts), covering the entire area with regrown stems and thereby keeping the soil from evaporation with the weeds simultaneous destruction. The plants' high leafiness (more than 60% of the total aboveground mass) and many root branches make it possible to assimilate a heavy metals significant amount from the soil and air.
Oil-contaminated soil is irrigated with a solution of PABA (para-aminobenzoic acid) at a concentration of 0.1-0.2%.

PABA is known as reparogen, it is considered as a genetically significant compound that stimulates regenerative processes in cells. Participates in the synthesis of folic acid, normalizes metabolism in connective tissue. In this technical solution, the damaged microflora of the soil is stabilized and restored using the PABA effect on the microflora.

PABA is a crystalline substance that is poorly soluble in cold water and dissolves well at 70-80°C. PABA has a wide effect on various taxonomic units of plants, animals, insects, microorganisms, is a growth factor for many types of bacteria, including those contained in the biological product Nikfan.

PABA - vitamin H - is a colourless crystalline substance that is used as a growth factor for microorganisms. Combining with enzymes, it increases the enzymatic catalysis energy at ontogenesis certain stages, thereby imparting additional resistance, plasticity and productivity to the genotype.

It is possible to revive the earth and restore its fertile layer (humus) with the bio compost help containing a microorganisms (Nikfan) symbiotic association. The "living systems" technologies use in sustainable human activities interests allows not only to solve the problems associated with the natural soil fertility restoration. The biological product Nikfan effective microorganisms, containing various microorganisms complex, represent a physiologically compatible stable community and complementary beneficial microorganisms responsible for the regeneration processes, giving the life force.

Microbiological fertilizer Nikfan is a lactic acid consortium aqueous solution consisting and photosynthetic bacteria capable of fixing atmospheric nitrogen, yeast, and organisms biologically active waste products.

This biological product use makes it possible to solve problems associated with natural soil fertility, prevent environmental pollution, and ensure soil water and air permeability to a depth of 60-80 cm.

Due to this biological product introduction into the toxic soil, the humus formation processes are accelerated several times within 2-3 weeks.

In contrast to the known techniques, the proposed object uses stevia plants (Stevia rebaudiana) as a plant origin sugar substitute. Stevia leaves are 10-15 times sweeter than sugar. Stevia contains many antioxidants - quercetin, rutin, minerals - calcium, phosphorus, potassium, zinc, chromium, magnesium, selenium, copper, as well as vitamins of group A, B, C, E. Also, it contains 53 active substances (in including stevioside), promotes the microorganisms revival and activity.

This mixture, with the PABA help sweetened with stevia, enhances the microorganisms activation and within 2-3 weeks the oil hydrocarbons amount in the soil is significantly reduced. The method (2-3 weeks) parameters are explained by the microorganisms' activation action in the soil with the introduced biologicals' help, dissolved in PABA in a mixture with the stevia plants sweetening. This speeds up the humus formation process.

After improving the fertility, an amaranth crop is sown that is resistant to soil toxicity and has high sorption properties to absorb heavy metals.
In the branching phase, to stimulate the amaranth growth, repeated watering is carried out in the same concentration, which simultaneously provides cleaning from petroleum products (table 3).

### Table 3. Oil-contaminated land remediation.

| Experiment variants                      | Concentration oil, g/kg | Oil reduction, % | Hydrocarbons mg/kg |
|-----------------------------------------|-------------------------|-----------------|--------------------|
| Control (no irrigation)                 | 66.4                    | -               | 2680               |
| Watering with a PABA aqueous solution, 0.1-0.2% | 57.0                    | 14.2            | 1812               |
| Watering with a water solution Nikfan (without stevia) | 48.2                    | 27.5            | 1620               |
| PABK watering + stevia + Nikfan         | 36.8                    | 44.1            | 860                |
Sowing amaranth with watering in the branching phase 42.5 36.0 1180
PABA + Stevia + Nikfan + Amaranth sowing 24.8 62.7 362

Analyzing the data obtained in the table, we can conclude that oil-contaminated soils irrigation with para-aminobenzoic acid mixed with stevia plant substances and the biological product Nikfan can significantly reduce the oil hydrocarbons content in the soil.

4. Conclusion
Oil-contaminated lands rehabilitation is carried out by sowing amaranth plants, zeolite-containing alanite clay, followed by sowing a legume-cereal mixture and plowing them in the flowering phase.

The use of plant waste of corn stalks, sunflower baskets in a mixture with zeolite-containing clays helps to reduce soil toxicity, reduces soil acidity, restores soil fertility.

On oil-contaminated soils, a recommendation effective method is to use paraaminobenzoic acid (PABA) in a mixture with 0.2% aqueous solution of the stevia plant and 2-3 weeks after watering, the amaranth crop is sown.

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