Abstract

Heavy metal pollution in agricultural soil is one of the most important environmental problem for the different scientists, recently years. Heavy metal contamination in the agricultural soil is not only pollution but it also has dangerous effect on wild life and human life. The solution of this pollution problem by using classical traditional physical and chemical methods is too expensive. But, phytoremediation method is using for removal of heavy metal from agricultural soils, recently. This method is cheaper than classical traditional physical and chemical methods. Kinds of phytoremediation method are phytoextraction, phytodegradation, phytostabilization, phytovolatilization, rhizodegradation, rhizofiltration, phytohydraulic control, vegetative cover systems, buffer strips and riparian corridors. These kinds of phytoremediation methods were evaluated in this study.

Keywords: phytoremediation, heavy metal, soil, pollution, plant

1. Introduction

Phytoremediation, which is derived from the words “phyto” (plant) and “remediation” (recovery) and has become a term in 1991, can be also defined as “bioremediation,” “botanical remediation” and “green remediation.” Phytoremediation is a term which is related to ecological remediation technologies that use plants as the main source. With this technology, organic and inorganic substances are removed from the contaminated area by using plants. The effects of this method can be observed in low polluted areas in a short time. The negative aspect is that in heavy contaminated areas the plants cannot be useful in a period of short time [1, 2].
The plants which are identified as metal hyper-accumulators and wild, are able to remove contaminant elements 10–500 times higher compared to the ones that are cultivated [3].

Phytoremediation method is ecological, does not need special equipment during application and provides a re-usable land. The root depths and climatic conditions play an important role in the efficiency of the system. First of all, the soil must be appropriate to the needs of the plant for the removal of the contaminants from the soil by the plant. The pH of the soil is one of the most important parameters. The pH levels of the area must be between 5.8 and 6.5 for the nutrient elements to be taken [4]. The absorption of the contaminants and their accumulation by the plants is presented in the Figure 1.

The nutrient element absorption is completed in three stages: (1) the transportation of the nutrients to the root circle and root surface; (2) the absorption of the nutrient ions into the roots; and (3) the transportation of the nutrient ions which entered into the root to the necessary parts by the transmission branches. There are two basic theories in the transportation of the nutrients to the root surface: “Intersection and Contact Change” and “Carbonic Acid Theory” [6].

Figure 1. Heavy metal absorption and accumulation in root and shoot of the plant [5].
2. Phytoremediation techniques

Phytoremediation, which has become more common in the last 10 years, is a passive technology which is related to soil recovery. Phytoremediation is the use of green plants in the removal of the contaminants from the area or in their recovery [7].

According to Salt et al. [8] phytoremediation techniques can be subcategorized as phytoextraction, phytodegradation, rhizofiltration, phytostabilization, phytovolatilization and rhizodegradation. Phytoremediation techniques are very effective in the sterilization of the areas that are medium-contaminated and have slight risk.

2.1. Phytoextraction (vegetal assimilation)

This technique is used in the absorption of the organic and inorganic contaminants by the roots and the sprigs of the plant. It is a valid method for the recovery of the contaminated areas, in which the plants that are able to absorb metals are chosen and the contaminants are removed from the soil with the harvesting or removal of the plant.

Because this technology’s application takes more time compared to other techniques, its application on heavy polluted areas is very hard. Also, a plant which grows in that ecosystem should be chosen. It should not be seasonal, because they will be harvested later. After they are harvested, they are burned in incinerator or exposed to another method with composition [9]. This method which is called phytomining, provides the opportunity for obtaining the mineral ores whose cultivation process is not economic. With this method, gold and nickel are re-gained in the USA [1, 10].

When the plants used in this method are compared to other plants, it can be observed that they can accumulate contaminant elements 100 times more. In this method Brassicacea, Euphorbiacea, Asteraceae, Lamiaceae and Scrophulariaceae and 400 other types are identified which can accumulate heavy metals. The residues of the harvested plants can be isolated by drying, burning, composting and recycling to biological metal minerals [11].

2.2. Phytodegradation (vegetal degradation)

Phytodegradation is a method in which organic contaminants are degraded by the compounds that are produced by plants through metabolic processes. Vegetal degradation can be applied to soil, clay, sediment and underground waters. The most advantageous aspect of the method is that the reduction and degradation occur inside the plant as a physiological process, and do not depend on microorganisms, while the emergence of toxic intermediate and end-use products, and the difficulty of their detection create a disadvantage [10].

The absorption of the organic compounds into the plant depends on the plant type, the residence duration of the contaminant element in the soil, and the soil’s physical and chemical form. The easily dissolved compounds are difficult to absorb. Plant enzymes are known to be able to degrade hazardous substances such as herbicides, munitions wastes and chlorinated solvents (trichloroethane (TCE)) [11].
2.3. Phytostabilization (root stabilization)

This method is used in the stabilization of soil. Phytostabilization plants are able to tolerate heavy metal levels and immobilize the metals through sorption, sedimentation, complexation or reduction of metal valences. The contamination factors in soil occur as a result of the immobilization of the contaminants around the plant roots, their accumulation by the roots, cohesion or sedimentation around the roots [12].

Wang et al. [13] conducted a research on the development and Cu absorption of corn plant (Zea mays L.) which is inoculated or non-inoculated by Acaulospora mellea, an arbuscular mycorrhizal fungus, by using different doses of Cu-applied pots in laboratory conditions. They concluded that the low absorption of the plants in the high concentrated Cu pots results from the soil’s pH value. They observed that the concentration and the structures of the organic acids in the soil such as malic acid, citric acid and oxalate acid were modified by the fungus. The researchers revealed that Acaulospora mellea is not suitable for the phytoextraction of copper by the corn plant; however, mycorrhizal plants are more applicable for phytoextraction because of their high capacity of Cu absorption in their roots.

On the other hand, contaminants’ transportation by wind, water erosion, washing out or soil dissemination can be prevented. In a system which is closely related to the plant’s root environment microbiology and chemistry, the plant is able to modify the contaminant factor’s form into non-resoluble or non-transported in water [1, 2].

2.4. Phytovolatilization (vegetal evaporation)

The root depth is very crucial in phytovolatilization. If it is about underground waters, the roots should be deep. To sterilize contaminated underground waters, the water can also be pumped to the ground to provide absorption for the surface roots. The most important aspect of this method is the transformation of the excessive toxic compounds (mercury contained compounds) into less toxic forms. However, the potential release of these hazardous and toxic materials into the atmosphere is a disadvantage [1]. The contaminants can be removed from the plant by transpiration or evaporation. As a well-known fact, water is carried from the roots to the leaves with the help of vascular system; therefore, the contaminants are released to the air through evaporation or volatilization. Poplar tree can be an example for this mechanism [9].

Ghosh and Singh [14] pointed out that some plants such as Brassica juncea and Arabidopsis thaliana can release heavy metals to the atmosphere with phytovolatilization by absorbing and transforming them into gas form.

Some types of trees such as Populus and Salix are often used in phytovolatilization because of their capacity to take contaminants with phytoremediation [15].

2.5. Rhizodegradation (the use of roots for degradation)

Rhizodegradation is the decomposition of the organic contaminants in soil surrounding the roots of the plants as a result of microorganism activities. There are amino acids, sugar, organic acid, sterol, fat acids, growing factors, nucleotide, flavanone and enzymes which are
released from the plant’s roots and affect the microbial activities in the surrounding area of the roots. The most important benefit of Rhizodegradation method is the dissolution of the contaminants in their natural environment [1, 2].

Pesticides (herbicide, insecticide), benzene, toluene, ethylbenzene, xylene (BTEX), total petroleum hydrocarbon (TPH), polycyclic aromatic hydrocarbons (PAH), surface active substances, chlorinated solvents (TCE, TCA), pentachlorophenol (PCP) polychlorinated biphenyls (PCB) can be exemplified as contaminants that can be dissolved with Rhizodegradation. Mint (*Mentha spicata* L.), red berry (*Morus rubra* L.), lucerne (*Medicago sativa* L.), and reedmace (*Typha latifolia* L.) are used in Rhizodegradation method [1, 4, 16, 17].

### 2.6. Rhizofiltration (the use of roots for filtration)

In rhizofiltration method, contaminants cling to the roots or absorbed by the roots in accordance with biotic and abiotic processes. During these processes, contaminants may be taken or transported by the plant. What is important is to maintain the immobilization of the contaminants in or on the plants. Later on, the contaminants can be taken from the plants with different methods. This method is applied to underground waters, surface waters and waste waters [4, 18].

Rhizofiltration is used to remove the radioactive substances or metals from the contaminated waters. The plants which are used in this method are directly planted on the contaminated soil and the contaminant’s adaptation is ensured. The plants are raised hydroponically in clean water instead of soil until they have a wide root system. The rooted plants are transported to the contaminated water source in order to make them adapt to their new environment. When the roots become saturated the plants are harvested. This method provides an opportunity for the use of terrestrial and aquatic plants. It is also used in basins, tanks, and ponds besides natural environment [8, 12].

### 2.7. Phytohydraulic control

Hydraulic control is a method which prevents and controls the accumulation and transportation of the contaminants by using plants. This method is applied to both underground and surface waters. The advantageous aspect of this method is the wide impact area because of the expansion of the roots without any artificial system. However, the instability of water absorption depending on season and climate is a disadvantage.

According to Pivetz [10], a 5-year-old *Populus* tree can absorb 100–200 liters of water in a day. A single salix tree’s amount of perspiration is claimed to be 5000 gallon of water in a day. Salix, hybrid populous and Eucalyptus can be used in this method. The Phytohydraulic control method is generally used in the dissolution of organic and inorganic water-soluble contaminants [1].

### 2.8. Vegetative cover systems

Vegetative cover is a method in which the contaminants are controlled by the long-term and self-growing vegetative system. Vegetative cover systems expand over or inside of the substances with environmental risks and require minimum care. Vegetative cover is generally set up as barriers that prevent the expansion of contamination [1].
Vegetative covers are applied to the contaminant area or to the surface around the units that spread the contaminants. This system is planned to be used in the USA as an alternative in covering solid waste storage areas considering it as a cheap ecosystem which minimizes surface erosion by regenerating itself [1, 4, 10]. However, the constant control of the necessary long-term maintenance of the cover system creates a disadvantage, because some plant types may dominate the others in the course of time [19].

2.9. Buffer strips and riparian corridors

Buffer strips and riparian corridors are systems in which the suitable plants are planted in stripes throughout stream rank in order to remove the contaminants in underground and surface waters that stream towards the rivers [4, 10]. This method prevents the water contaminants to spread and interfuse into ground water. The studies conducted in Canada revealed that this system removes soil erosion up to 90%, and herbicides up to 42–70% [20]. Buffer strips method is mostly used in many countries in the removal of contamination caused by fertilizers and pesticides. Populus is the most used tree in this method [1].

This method is mainly used in the removal the contaminants in underground and surface waters that stream towards the rivers by plantation in stripes throughout stream rank. Therefore, the water contaminants are prevented to spread and interfuse into ground water. On the other hand, erosion is controlled and sediment is reduced. The studies have shown that this system removes sediment in water up to 71–90%, nitrogen up to 67–96%, phosphorus up to 27–97% pesticides up to 8–100% and fecal coliforms up to 70–74% [19, 20].

3. Phytoextraction of heavy metals with Canola in model field

We performed the phytoextraction with canola in the model fields to estimate ant effectiveness.

3.1. Experimental conditions

The experiment is conducted on the research fields of Faculty of Agriculture, Namik Kemal University, Turkey, according to the randomized block design with 3 replicates. 100 mg/kg Co, Cr, Ni and Pb ions are taken from CoSO₄, Cr(NO₃)₃, NiSO₄ and Pb(NO₃)₂ compounds and applied to the soil as contaminants. There are 51 parcels with 4 contaminants (CoSO₄, Cr(NO₃)₃, NiSO₄ and Pb(NO₃)₂) × 4 chelate doses (EDTA) (0, 5, 10 and 15 mmol/kg) × 3 replicates + 3 control. The control parcels in which the contaminants and chelate are not applied are organized as three replicates. Each parcel in the test are sized as (3 × 1.2 m): 3.6 m² including four rows (for instance for lead element PbEDTA₀, PbEDTA₅, PbEDTA₁₀ and PbEDTA₁₅). The row distance in each parcel is 30 cm, the distance between each parcel is 0.5 m and between each block is 1.5 m. The height of each block is 3 m and the width is 31.2 m (there are 4 parcels in each block). Therefore, a whole block is of 93.6 m² and the total test area is of 284.6 m², including the distances between the blocks (Figure 2).
3.2. Physical and chemical characteristics of the test soils

The physical and chemical characteristics of the samples taken from the testing field are presented in Table 1. According to the table, the soil’s pH is neutral, low lime and has insufficient organic matter. Its available phosphorus content is sufficient as well as the exchangeable potassium. The amount of available iron is average, available copper and manganese is sufficient, and available zinc is insufficient. Also, the testing soil is classified as clay in terms of texture [21, 22].

| Soil properties       | Unit     | Values |
|-----------------------|----------|--------|
| pH (soil: water=1:2.5)|          | 6.81   |
| EC (x10⁶)             | dS/m     | 128.3  |
| CaCO₃                 | %        | 2.40   |
| Organic matter        | %        | 1.88   |
| P₂O₅                  | kg/da    | 11.42  |
| K₂O                   | kg/da    | 25.32  |
| Fe                    | mg/kg    | 3.46   |
| Cu                    | mg/kg    | 0.63   |
| Zn                    | mg/kg    | 0.40   |
| Mn                    | mg/kg    | 5.72   |
| Clay                  | %        | 42.98  |
| Silt                  | %        | 25.44  |
| Sand                  | %        | 31.58  |
| Texture class         |          | C      |

Table 1. Some physical and chemical characteristics of the testing soil [23].
3.3. The amount of heavy metal (Cr, Co, Ni and Pb) before the test and after the incubation

The extractable Cr, Co, Ni and Pb contents of the testing fields are identified before the heavy metals are applied to the soil and they are presented in Table 2. 100 mg/kg Cr, Co, Ni and Pb are applied to the testing field and left to incubation for a month. The extractable heavy metal contents are determined after the incubation. T-test is applied to the results and the standard error values are given in Table 2.

When the Table 2 is examined, it can be observed that the amount of extractable heavy metal contents before the test is acceptable and does not have any contaminant characteristic [1]. A remarkable increase is observed as a result of 100 mg/kg Cr, Co, Ni, Pb heavy metal application upon one-month incubation. These increases are determined as 1% significant statistically.

3.4. The effects of EDTA applications on heavy metal contents (Cr, Co, Ni, Pb) of the root and shoot of the plant

The effects of increasing doses of EDTA applications on heavy metal contents (Cr, Co, Ni, Pb) of the root and shoot of the plant are presented in Table 3.

The amount of Cr in the roots and shoot of the canola plant which grows on Cr-applied fields rapidly increased after 0 mmol/kg EDTA dose and reached the highest level with 15 mmol/kg EDTA dose. These increases were determined as 1% significant statistically.

Similar to the Chrome element, the amount of cobalt in the root and shoot of canola plant which grows on the cobalt-polluted field increased with EDTA applications. These increases were determined as 1% significant statistically (Table 3).

The amount of nickel in canola plant which grows on the field that has been polluted with nickel increased with EDTA applications, and the highest levels were achieved on the parcels which were applied with 15 mmol/kg EDTA dose. It can be concluded that with the increasing doses of EDTA, the Ni concentration in the roots and shoot of the plant has also increased. These increases were determined as 1% significant statistically and various groups are formed in Duncan multiple comparison test (Table 3).

The amount of Pb in canola plant which grows on the field that has been polluted with Pb increased with EDTA applications, and the highest levels were achieved on the parcels which

| Heavy metal | Before polluting | After polluting |
|-------------|------------------|-----------------|
| Cr          | 0.10 ± 0.01"     | 5.80 ± 0.07"    |
| Co          | 0.08 ± 0.05"     | 2.25 ± 0.02"    |
| Ni          | 0.95 ± 0.05"     | 6.20 ± 0.13"    |
| Pb          | 0.93 ± 0.01"     | 7.52 ± 0.04"    |

" p<0.01.

Table 2. The extractable heavy metal contents before and after the application of the contaminants to the testing soil.
were applied with 15 mmol/kg EDTA dose. It can be concluded that with the increasing doses of EDTA, the Pb concentration in the roots and shoot of the plant has also increased. These increases were determined as 1% significant statistically and various groups are formed in Duncan multiple comparison test (Table 3).

In Table 3, the amount of heavy metals (Cr, Co, Ni and Pb) in canola plant’s roots and shoot, Duncan multiple comparison test and standard error values are presented. According to Table 3, the amount of heavy metals in the roots is higher than the heavy metals in shoot.

According to Table 3, on the fields which are applied with 0 mmol/kg EDTA doses, the amount of Cr, Co, Ni and Pb heavy metals in the roots and shoot of the canola plant is lowest and the highest heavy metal level can be detected in 15 mmol/kg EDTA dose. The results are equal to other research results on this subject. The researchers have explained that the solubility and absorption of the heavy metals get easier for the plant with the increasing doses of EDTA application [27–31].

According to the results of this research, EDTA applications should be conducted by growing hyper-accumulator plants on the soils in order to decrease Cr, Co, Ni and Pb heavy metal contamination under the toxicity levels. Because, it has been proved that with the increasing doses of EDTA application some heavy metals (Cr, Co, Ni and Pb) can be removed from soil with phytoremediation (Phytoextraction) method.

| EDTA application | Chrome (Cr) | Cobalt (Co) |
|------------------|-------------|-------------|
|                  | Root        | Shoot       | Root | Shoot |
| Control          | 2.75 ± 0.57a | 4.23 ± 0.58a | 1.95 ± 0.02a | 2.18 ± 0.04a |
| 0 mmol/kg        | 14.70 ± 007b | 8.25 ± 0.56a | 13.30 ± 0.08b | 11.56 ± 1.39b |
| 5 mmol/kg        | 29.16 ± 0.58c | 26.42 ± 0.21b | 28.60 ± 1.50c | 24.45 ± 0.59c |
| 10 mmol/kg       | 55.12 ± 0.99d | 40.45 ± 1.05c | 51.40 ± 2.61d | 39.12 ± 1.14d |
| 15 mmol/kg       | 70.50 ± 1.92e | 52.20 ± 1.50d | 75.40 ± 1.56e | 45.20 ± 0.60e |

| Nickel (Ni) | Lead (Pb) |
|-------------|-----------|
| EDTA application | Root | Shoot | Root | Shoot |
| Control      | 4.76 ± 0.11a | 5.83 ± 0.63a | 3.78 ± 0.45a | 4.96 ± 0.56a |
| 0 mmol/kg    | 24.43 ± 0.13b | 13.12 ± 0.97b | 24.50 ± 0.97b | 18.90 ± 0.54b |
| 5 mmol/kg    | 49.65 ± 0.34c | 37.60 ± 0.56c | 41.40 ± 0.66c | 35.20 ± 0.05c |
| 10 mmol/kg   | 77.80 ± 0.60d | 61.40 ± 0.28d | 87.80 ± 0.90d | 63.14 ± 1.14d |
| 15 mmol/kg   | 85.30 ± 1.01e | 65.10 ± 0.057e | 95.40 ± 0.17e | 70.12 ± 0.01e |

*Each heavy metal element, root and shoot is examined separately with three replicates.

Table 3. The effects of EDTA applications on the amount of heavy metals (Cr, Co, Ni and Pb) in the roots and shoot of canola plant that were grew (mg/kg) [23–26].
4. Conclusion

According to the field experiment results, the amount of Cr, Co, Ni and Pb heavy metals, removed from the soil by the canola plant, increased with the increasing of EDTA applications. This increases and decreases were found statistically significant at the level of 1%. It was an expected rate, because application of chelates like EDTA on soil accelerates solubility of some heavy metals (Cr, Co, Ni and Pb) in soils and their absorption by plants. It was relatively expensive and limited in use to remove some heavy metals like Cr, Co, Ni and Pb which cause pollution in agricultural lands and are particularly results of industry-related human activities, from the soil by classical physiochemical methods.

Therefore, it becomes gradually important to remove Cr, Co, Ni and Pb heavy metals naturally from the agricultural lands by increasing its mobility in soil, with the help of various hyper accumulator plants such as canola, which are relatively inexpensive and practical in use but changeable concerning the concentrations and types of heavy metals.

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