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Use of vetiver (*Vetiveria zizanioides*) in remediation of cyanide soil contamination

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ABSTRACT

The purpose of this greenhouse study was to assess the vetiver (*Vetiveria zizanioides*) in terms of the capacity to tolerate and accumulate cyanide (KCN) from contaminated soils of varying physicochemical properties. The toxicity of potassium cyanide (KCN) to grass vetiver (*Vetiveria zizanioides*) was tested. At the end of test, almost all cyanides had vanished from soils. Also, the *in vivo* capacity of *Vetiveria zizanioides* to remove cyanide was evaluated. For cyanide adsorption onto *Vetiveria zizanioides*, various parameters, affecting the removal rate, such as pH (6-8), and contact time (3-9months) were evaluated. The effect of pH on cyanide complex ion uptake rate and capacity of *Vetiveria zizanioides* was studied at 4-8 mg kg⁻¹ initial cyanide concentration. Cyanide adsorption was increased with increasing soil pH, water content and contact time. At an optimum pH of 8, over 85% removal of 8 mg kg⁻¹ cyanide was obtained for *Vetiveria zizanioides* after 9 months of contact time. Data from the experiment were analyzed with statistical software SPSS. The removal of cyanide by vetiver might be useful in cleanup of soil from gold and silver mining.

Key words: *Vetiveria zizanioides*, Cyanide removal, Soil, water content, pH

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1. INTRODUCTION

Cyanide is widely used in extraction of gold and silver. Cyanide compounds are released into environment by various industries, including the mining industry, to assist in the extraction of both precious and non-precious metals from rock, hardening of metals, electroplating, chemical synthesis photography, pharmaceuticals, coal coking, ore leaching, plastics and etc (1-3). Cyanides are generally classified as free cyanide (i.e. cyanide anion and hydrogen cyanide) and metalcyanide (i.e. weak acid dissociable cyanide). Among cyanides, free cyanide has been known as the most toxic species because of hydrogen cyanide which can cause serious respiratory and neurological effects consequent to short or long-term exposure (2, 4-14). Cyanide also tends to react readily with many other chemical elements, producing a wide variety of toxic and cyanide-related compounds (4). Cyanide is included in the CERCLA¹ priority list of hazardous substances and has adverse health effects on human (15). Cyanide's toxicity results from its propensity to bind to the iron in cytochrome c oxidase, interfering with electron

transport and resulting in hypoxia. Cyanide is lethal to human (2, 5-7). Cyanide belongs to nitril group and has adverse effects on health because this group has a harmful effect on respiratory system (5). Soils contaminated with cyanide represent a serious environmental and human health problem due to the possible transfer to the food chain and need an effective technological solution. Chemical and biological methods mentioned in the present literature can be used for removing the heavy metal cyanide complex ions from soil (2, 4-14, 16-19). Natural degradation of cyanide is one of the primitive methods used to destroy the cyanide in industrial wastewater in gold and silver industries. Treatment of cyanide containing wastes alkaline chlorination, oxidation process, electrolytic, decomposition, ozonation, etc.(4, 20, 21). Although chemical and physical mechanisms can be employed to degrade cyanide and its related compounds, they are often expensive and complex to operate. Phytoremediation is a green and sustainable technology which refers to employing plant species for environmental cleanup and it has often been offered as a potentially inexpensive and

environmentally friendly alternative to conventional processes (12, 18, 22). Phytoremediation is more cost-effective and environmentally-sustainable than alternative mechanical or chemical methods for removal of hazardous contaminants from the soil in the last few years (23, 24). Plant selection is critical for phytoremediation applications and is typically based on a number of factors, including the site, cost comparison with other remedial technologies, contaminants, bioavailability, and soil type (25). Vetiver grass (*Vetiveria zizanioides*) could absorb substantial amounts of Cd, Hg and Pb in waste water. Vetiver grass has high tolerance to Se, Al, Mn, Ni, Cd, Cr, Pb, Hg, As and Zn in the soil (25, 26). This species is an efficient, low cost, and long-term remedial option for phytoremediation. Five different woody plants (willow, birch, poplar, rose) were all able to sorption free CN (HCN and CN⁻) from a solution (9). Vetiver grass (*Vetiveria zizanioides*) is one of the most widely distributed grass species in south and southeastern Asia that plays a considerable promise for soil and water conservation as well as phytoremediation applications because of its robust root system and tolerance for adverse environmental conditions (27). Vetiver grass (*Vetiveria zizanioides*) is a tall (1–2 m) fast growing perennial grass with quick regeneration ability. It has a long (3–4 m), massive and complex root system which can penetrate to the deeper layers of the soil and it is developed as a suitable plant for solving many of the environmental problems (24, 25, 28-30). Vetiver grass technology will become one of the leading biological systems of environmental protection in the 21st century (30). There are reports on the use of this plant for phytoremediation of soils contaminated with 2, 4, 6-trinitrotoluene (11, 12), petroleum (23), benzo[a]pyrene (6) nuclear waste (25). However, there was no report about using vetiver grass for phytoremediation of soil contaminated with cyanide. This study was intended to provide baseline information on the capacity of vetiver grass to tolerate and accumulate cyanide from contaminated soils of varying physical and chemical properties. The principal objective of the present study was to determine the efficacy of Vetiver grass (*Vetiveria zizanioides*) for removing cyanide.

2. MATERIALS AND METHODS

2.1. Soil preparation

Soils samples were collected from the surface (0–30 cm) experimental stations at Applied Geological Research Center of Iran. Soil samples were air-dried, crushed to pass through a 3 mm diameter sieve, and mixed thoroughly. The physical and chemical characteristics of the soils are showed in Table . The electrical conductivity (EC) of the soil was measured by a conductivity meter. Soil pH was measured by pH meter.

2.2. Planting vetiver grass

The soil samples (10 kg) were placed in plastic pots (30 cm diameter and 50 cm height). The moisture level of the soil was held to near field water capacity (35.6%) and equilibrated for three weeks. The seedlings of vetiver grass (*V. zizanioides*) were selected and pruned (the shoots were 25cm high and the roots 8 cm long), and then transplanted into the vases in April 2013. The pots were irrigated daily to 65% of the field water capacity.

2.3. Pot experiment

Each soil was air-dried for one week before use in the pot experiment. Five weeks after the seedlings of vetiver grass were transplanted in the pots, cyanide was adjoined to the surface of the soil in the spiked pots at rates 4- 8 mg /kg in a greenhouse setting .A proper amount of KCN salt was dissolved in distilled water to obtain the following KCN solutions 4-8 ppm. Cyanide ions in water was measured according to the ISO 14403:2002 standard method (31). The cyanide adsorption capacity of the media was evaluated in batch cyanide adsorption experiments at three final pH values 6.5-8.5 in 3, 6 and 9 months with an initial cyanide concentration of $C_0 \approx 4-8$ ppm. These concentrations were chosen as the lowest toxic levels in soils (4). Higher concentrations of cyanide could cause toxicity symptoms, including death of the vetiver plants during experiments. Following the application of cyanide, the soil was irrigated to 60% of field water capacity on a daily basis. All of the vase experiments were carried out in a greenhouse under natural light conditions. Air temperature ranged between 18 to 36 °C. Each soil and vetiver grass treatment was replicated 3 times, and the vases were in a completely random block design. Unplanted pots were added as controls. Three replicates were prepared for each sample and vetiver was grown from April to October, 2013. The concentration of cyanide in soil samples was determined by spectrophotometry according to the ISO 17380:2013 standard method (29).

2.4. Plant harvest

There were three replicates, giving a total of 30 pots in a fully randomized design in a greenhouse at the applied geological research center (GRCIR) of Iran. To ensure the accuracy and precision of the analysis reagent blanks and analytical duplicates were used one at a time. Plants were grown under glasshouse conditions from April to June, 2013. At 3rd, 6th and 9th month after treatment, seedlings were carefully moved from the vases then 1.00 gram of the samples were dried at 70 °C for 72 h, into a 25 mL centrifuge vial by adding 10 mL of 1 N NaOH. Then, it was shaken for 10 min, and immediately filtered into a 200 mL volumetric flask. Afterwards, it was diluted with reagent water while protected from light to determine available and total cyanide (32).

Table 1. The physicochemical characteristics of the soils used in the study Soils used in the pot experiments

Physicochemical properties	Soil1(low pollutant)	Soil2(average pollutant)	Soil3(high pollutant)
pH	6.58	7.5	8.53
EC(ds/m)	3.97	5.52	6.2
%N	0.248	0.202	0.213
P(ppm)	0.24	30	21
%sand	71	89	63
%silt	18	6	20
%clay	11	5	17

2.5. Statistical analysis

Statistical analyses of the experimental data, such as correlation coefficients and significant differences, were performed using statistical software SPSS.

3. RESULTS AND DISCUSSION

Although, the growth index was severely affected, vetiver grass was capable of withstanding a soil cyanide concentration of 8mg kg⁻¹ and surviving to the end of the assay. In addition, no symptoms of contamination were detected in the plants by simple observation. The experiment did not discriminate between these but data

show that vetiver roots enhanced the removal of cyanide from the contaminated soil, especially under high pH conditions. Vetiver plants would be suitable for phytoextraction of cyanide in mining industry, possibly for highly contaminated Superfund soils. The cyanide removal efficiency of vetiver grass will depend on the soil physicochemical properties. Soil properties had a major impact on growth and cyanide accumulation in vetiver plants. Nitrogen deficiency may increase the absorption and decomposition of iron-CN complexes. Cyanide removal efficiency of vetiver grass was calculated as follows (equation 1):

$$\text{Cyanide removal efficiency (\%)} = \frac{\text{Total cyanide in vetiver tissue(mg)} \times 100}{\text{Total Cyanide in column(mg)}} \tag{1}$$

The effect of water content, pH and contact time in removal of cyanide by vetiver grass yield is shown in Figures 1, 2 and 3. The effect of pH on cyanide complex ion uptake rate and capacity of *Vetiveria zizanioides* was studied at 4-8 mg kg⁻¹ initial cyanide concentration. The effect of pH was studied by varying the pH from 6 to 8. As the pH increases to 8, the amount of cyanide uptake also increases in vetiver grass. Increase in biosorption was

observed at higher pH. Free cyanide form depends on water pH. With increase of pH when the groundwater is exposed to sunlight before consumption, free CN can be formed. Increase of soil pH can be urgent for the plants to survive in the generally very acidic soil. The variation of adsorption rate and equilibrium uptake with initial pH is given in Figure 1.

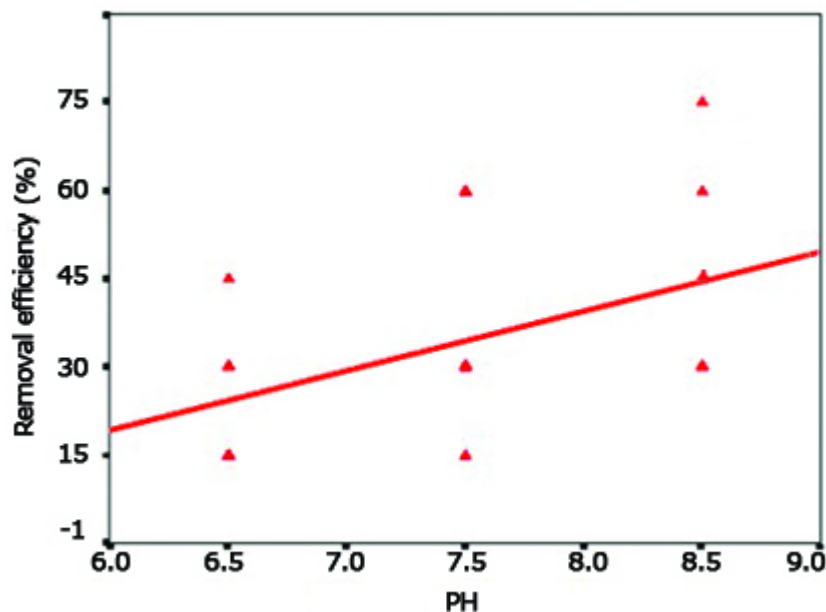


Figure 1. Effect pH in removal cyanide on vetiver grass

The equilibrium sorption capacity of the biomass for cyanide complex ions increased with an increase in initial

cyanide complex ion concentration up to 8 mg kg⁻¹. When *Vetiveria zizanioides* grown soil contaminated with cyanide at concentrations up to 4mg/L as CN, *Vetiveria zizanioides* showed no phytotoxic effects. Cyanide removal efficiency decreased with cyanide concentration in the soil. Removal of cyanide on vetiver grass increased with water contact, and contact Time Total cyanide

accumulation in shoot and root tissues after 3 and 6 and 9 months are shown in Figure 3. Vetiver accumulated substantial amounts of Cyanide in both shoot and root tissues; however, accumulation was higher in root tissue in all soils, and increased with increase in cyanide concentration. Figure 2 shows connection residual cyanide in the vetiver plants were harvested with time.

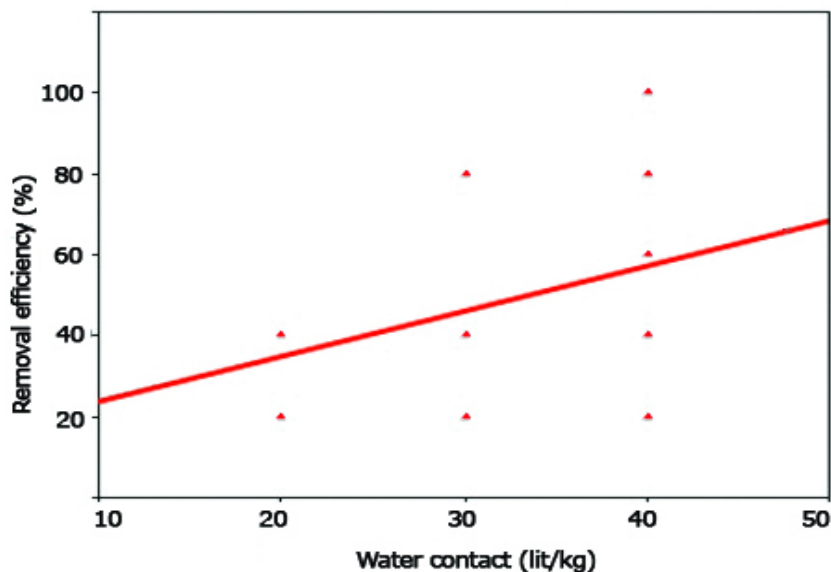


Figure 2. Effect of water contact in removal of cyanide by vetiver grass

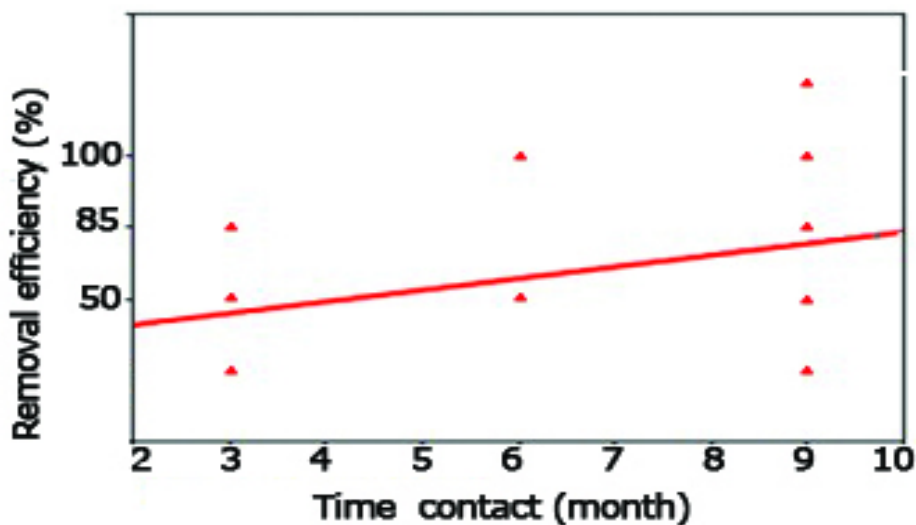


Figure 3. Effect of contact Time in removal of cyanide by vetiver grass

Significant differences were found in the cyanide concentrations in soils between the initial and final time periods. As expected, the highest amount of cyanide was lost in final time period (9 months). Phytoremediation effectively removes cyanide pollutants. C.M. Kao et al and C.Y. Chen et al and Sabatini et al used Several microorganisms (bacteria, fungi) to degrade cyanide and cyanide complexes (2, 4-14, 16, 18, 19, 22, 24, 28, 29, 33). We compared their studies with phytoremediation Vetiver and concluded that phytoremediation treatment methods are less expensive and environmental friendly. The microorganisms responsible for cyanide degradation are bacteria and fungus. If cyanide is in high concentrations, reduced bacterial activity occurs (13). Carbon has been

known as a limiting factor in the microbial degradation of cyanides of industrially contaminated soils (13). A variety of pollutants other than cyanide compounds in wastewater cause inhibitory effect on the growth of microbes, which in turn result in the decrease in biodegradation. M. Larsen et al. suggest that *Salix viminalis* cyanide has a beneficial role in treating cyanide known drawback and is a desirable solution of treating environmental sites contaminated with cyanide, Levels of .5mgKCNL.1 in hydroponic solution were toxic to basket willows (*Salix viminalis*) (10).

4. CONCLUSION

Cyanide is widely used and its uses include production of paints, silk, pharmaceutical products, insecticides, gold and

silver extraction, ore and metal degreasing so that CN has also made it an important environmental contaminant. With vetiver phytoremediation, the long and dense root system of vetiver, can absorb heavy metals from the deep soil layers, then transfer them to aerial part for harvest and thus reduce the metals concentration in soil. This study was performed as a laboratory experiment. Vetiver plants were used to investigate the uptake of cyanide. Experiments were performed as a function of pH, initial cyanide ion concentration and water and time contact. The experimental results showed that *Vetiveria zizanioides* has a considerable potential for uptake of cyanide complex ions from soil. Cyanide was not toxic to *Vetiveria zizanioides* and the transpiration-driven movement of CN into roots may prevent movement of CN to groundwater. Biosorption by *Vetiveria zizanioides* can be proposed as an alternative to more costly methods such as biological treatment, activated carbon adsorption, solvent extraction and chemical oxidation for removal of cyanide complex ions from soil. From the results obtained, it is possible to conclude that in principle, vetiver could be successfully used for remediation of cyanide-contaminated soils.

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CONFLICT OF INTEREST

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