Citric Acid and EDTA as chelating agents in phytoremediation of heavy metal in polluted soil: A review

by Sri Sumiyati

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Citric Acid and EDTA as chelating agents in phytoremediation of heavy metal in polluted soil: a review

Y C Shinta[1*], B Zaman[2], S Sumiyati[2]

[1]Master Program of Environmental Engineering, Diponegoro University, Semarang, Indonesia
[2]Departement of Environmental Engineering, Diponegoro University, Semarang, Indonesia

yesiclarashinta@gmail.com

Abstract. The application of metal chelating agents in phytoremediation has been shown to increase plant efficiency for heavy metal uptake in phytoextraction significantly. EDTA is a famous chelating agent used in phytoextraction. However, future use of EDTA is likely to be limited to ex-situ conditions where leachate control can be achieved, so there are limitations to its use that need to be studied. So that many phytoremediation studies have been carried out on organic chelating agents that are not expected to be harmful to the environment, one of which is Citric Acid. The purpose of this review is to compare commonly chelating agents, namely: EDTA as synthetic and Citric Acid as a natural matter for phytoremediation in polluted soils. This review also discusses the ability of Citric Acid and EDTA on phytoremediation, their effect on soil physiology and soil microbiology, advantages and disadvantages of each on the prospects of phytoremediation. EDTA can increase phytoextraction better than Citric Acid but can increase the risk of groundwater pollution because EDTA is difficult to degrade by the environment. In contrast, Citric Acid has been shown to increase phytoextraction, phytostabilization and harmless to the environment.

1. Introduction

Phytoremediation uses plants to remove pollutants in soil by absorbing pollutants and accumulating them in the shoots of plants or by stabilizing pollutants using microorganisms in the roots. Phytoremediation also includes the ability of plants to remove contaminants from groundwater and the ability to remove airborne contaminants.

Phytoremediation effectively removes pollutants at a lower cost than conventional treatment, can be applied to large areas, and does not cause harmful side effects to the environment. In recent years, phytoremediation using plants called hyperaccumulators has achieved remarkable results, successfully recovering contaminated land on a large scale. However, the long remediation period and the amount of biomass cause limitations in the practical application of phytoremediation [1]. Currently, there are four main Phytoremediation strategies for extracting metals from soil: (i) use of natural hyper-accumulators, (ii) increased metal uptake by high-biomass plants through the addition of chemicals (e.g., chelating agents) to the soil, (iii) phytovolatilization (Se, As, Hg), and (iv) enhancement of plant phytoextraction capability through genetic engineering.

This review discusses the role of chelating agents in phytoremediation to absorb pollutants from the soil to the shoots. In the field practice, hyperaccumulator plants grown in a new environment require adaptation to metal stress. Chelating agents are needed to inhibit the formation of ROS and stimulate root exudates so that plants can survive, and most importantly, chelating agents can increase the ability of heavy metals in the soil to be absorbed by plants.

Several types of chelating agents are used in phytoremediation research, including EDTA, HEDTA, DTPA, CDTA, EGTA, EDDHA, HEIDA, EDDS, NTA HBED, and Citric Acid [2]. The most commonly used chelating agents are EDTA, a synthetic chelating agent, and Citric Acid, a natural chelating agent [3]. EDTA is a synthetic chelator that is a versatile driving agent that can form four or six bonds with metal ions. Citric Acid ($C_6H_8O_7.H_2O$) is a natural source of organic acids, where pure Citric Acid is easily soluble in water, colourless and solid at room temperature. Citric Acid is known to be non-toxic and is considered a Generally Recognized As Safe (GRAS) compound.

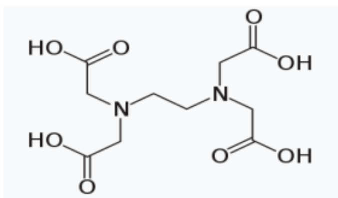

Figure 1. EDTA molecular structure.

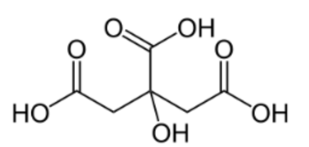

Figure 2. Citric Acid molecular structure.

2. The ability of Citric Acid and EDTA as chelating agents in Phytoremediation

Many researchers have explored the use of chelators for phytoremediation. Chelators can increase the absorption of heavy metals by increasing the bioavailability of metals by forming HMs-Chelator complexes. In the use of EDTA, the binding of heavy metals (HM) by EDTA to HM-EDTA occurs. The HM-EDTA complex ligand formation occurs in soils with 5.2 to pH 7.7 [4]. In Citric Acid, acidification of the soil using Citric Acid has increased phytostabilization and phytoextraction [5]. Furthermore, the following summary of the ability of Citric Acid and EDTA as metal chelators in phytoremediation from previous researchers is shown in table 1.

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Table 1. The ability of Citric Acid and EDTA as chelating agents in phytoremediation.

No	Author		Plants	Result: EDTA	Result: Citric Acid
1	Chigbo & Batty, 2013	[7]	*Medicago sativa*	34% Cr removal in soil compared with control plants (without chelator)	44% Cr removal in soil compared with control plants (without chelator)
2	Chigbo & Batty, 2015	[6]	*Zea mays*	Maximum Cu accumulation Cu accumulation on shoot 28.58 ug/kg Cu accumulation on root 25.41ug/kg Maximum Pyrene accumulation: Pyrene accumulation on shoot 16.57 ug/kg Pyrene accumulation on root 20.23 ug/kg	Maximum Cu accumulation Cu accumulation on shoot 12.39 ug/kg Cu accumulation on root 26.44 ug/kg Maximum Pyrene accumulation: Pyrene accumulation on shoot 8.60 ug/kg Pyrene accumulation on root 29.59 ug/kg
3	Zhang et al., 2018	[8]	*Moso bamboo*	Maximum Cu accumulation by 10 mmol/kg EDTA Accumulation on shoot 18.6 mg/kg Accumulation on root 271.5 mg/kg Maximum Pb accumulation by 10 mmol/kg EDTA Accumulation on shoot 7.4 mg/kg Accumulation on root 102.1 mg/kg	Maximum Cu accumulation by 10 mmol/kg CA Accumulation on shoot 15 mg/kg Accumulation on root 150 mg/kg Maximum Cu accumulation by 10 mmol/kg CA Accumulation on shoot 2 mg/kg Accumulation on root 40 mg/kg
4	Aghelan et al., 2021	[9]	Amaranthus caudatus L.	Maximum Pb accumulation Accumulation on shoot 0.03 mg/kg Accumulation on root 0.74 mg/kg	Maximum Pb accumulation Accumulation on shoot 0.02 mg/kg Accumulation on root 0.64 mg/kg
5	Turgut et al., 2004	[10]	Helianthus annuus	On application of 0.3 g/kg EDTA Maximum Cr accumulation on root = 0.223 mg/l Maximum Ni accumulation on shoot = 0.052 mg/l	On application of 3 g/kg Citric Acid Maximum Cr accumulation on root = 0.158 mg/l Maximum Ni accumulation on shoot below 0.01 mg/l
6	Mohammadi et al., 2021	[11]	okra (Abelmoschus esculentus L.)	On application of EDTA 1mM: Maximum Hg^{2+} in root 2.11 µg/g Maximum Hg^{2+} in shoot 2.35 µg/g	On application of CA 1mM: Maximum Hg^{2+} in root 1,98 µg/g Maximum Hg^{2+} in shoot 2.28 µg/g

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No	Author		Plants	Result	
				EDTA	**Citric Acid**
7	Han et al., 2018	[12]	Iris halophila Pall	TF (Shoot/root) **Pb** applicated by EDTA 2 mmol/kg is 0.092	TF (Shoot/root) **Pb** applicated by Citric Acid 2 mmol/kg is 0.091
				TF (Shoot/root) **Cu** applicated by EDTA 2 mmol/kg is 0.055	TF (Shoot/root) **Cu** applicated by Citric Acid 2 mmol/kg is 0.053
				TF (Shoot/root) **Cd** applicated by EDTA 2 mmol/kg is 0.529	TF (Shoot/root) **Cd** applicated by Citric Acid 2 mmol/kg is 0.560
				TF (Shoot/root) **Zn** applicated by EDTA 2 mmol/kg is 0.348	TF (Shoot/root) **Zn** applicated by Citric Acid 2 mmol/kg is 0.417
8	Gul et al., 2020	[13]	Pelargonium hortoum	Maximum Pb accumulation	Maximum Pb accumulation
				Accumulation on shoot 88% higher than control plant	Accumulation on shoot 80% higher than control plant

From the summary of table 1, the application of EDTA proved effective in accumulating various types of metals in plant shoots. When compared with control plants, the addition of EDTA in plants showed higher heavy metal removal than control plants (without a chelating agent). In Cu removal using Zea mays, EDTA application could accumulate Cu in stems better than Citric Acid, but Citric Acid showed higher Cu accumulation in roots than EDTA application and control plant. Based on a study of Pb absorption using Amaranthus caudatus L, EDTA increased metal uptake in shoots higher than Citric Acid. However, in the study of Cr Removal using Citric Acid and EDTA, Citric Acid had a higher Cr removal ability than control plants and EDTA by Medicago sativa. Meanwhile, the application of EDTA to Helianthus annuus for Ni removal showed the highest Ni accumulation in shoots compared to control plants and the use of Citric Acid. The conclusion from the above research is that the ability of EDTA as a chelating agent in phytoremediation is optimal in increasing phytoextraction, while Citric Acid is effective in phytostabilization.

2.1. The effect of EDTA and Citric Acid on soil physiology

Based on the study of the effects of two common chelators: EDTA and Citric Acid, on soil physical properties (such as soil structure, porosity and soil moisture), both chelators will increase root growth, especially in the use of Citric Acid. The addition of Citric Acid to heavy metal contaminated soil can improve soil physical properties [14]. It is related to root growth which the administration of Citric Acid stimulates. Root growth can compact the soil and reduce soil porosity in sandy soils. On the other hand, application to low porosity soil will increase soil porosity so that the aeration process for soil organisms can occur. The presence of root growth stimulated by chelating agents, both Citric Acid and EDTA, had a positive effect on the physical properties of the soil.

2.2. The effect of EDTA and Citric Acid on soil chemical properties

The effect of chelator on soil chemical properties in phytoremediation relates to pH's effect on heavy metal uptake. Research has found that changes in pH will affect the absorption of heavy metals in plant roots. pH above 8 causes the release of heavy metals in the soil, so bioavailability decreases and heavy metals that plants can absorb also decrease. When the pH is too low, the absorption of heavy metals by plants is not practical. The ability of metal absorption increases when the pH is above 2 and decreases after the pH is above 8 [15]. Acidification of the soil at the roots increases the absorption of metals. The addition of organic acids (Citric Acid) caused a pH decrease, thereby increasing the bioavailability of metals. Meanwhile, the addition of EDTA did not cause a change in pH because EDTA has a neutral pH.

2.3. The effect of EDTA and Citric Acid on soil microbiology

The effect of chelating agents on soil microorganisms is vital to study because the soil is the place for microorganisms to grow and reproduce. Microorganisms play an essential role in increasing soil fertility, including the degradation of heavy metals in soil. Increase the population of microorganisms [16]. In the 90 days of experiments, to determine the effect of Citric Acid addition on the population of microorganisms in polluted soil, there was an increase in the number of microbes with a rhizome effect value of 9.1. [17] Citric Acid stimulates the release of exudate by the roots naturally and creates a suitable environment for microorganisms. In addition, Citric Acid stimulates root growth. From comparing study results between EDTA and Citric Acid, Citric Acid absorbs more heavy metals in roots than EDTA. Besides that, Citric Acid promotes root growth better than EDTA. Increased root growth will increase the absorption of organic substances and heavy metals, but it can also increase aeration so that the microorganisms found in the roots can multiply. Citric Acid is an organic acid in the soil that can be decomposed by microorganisms quickly because Citric Acid is used as a food source for microorganisms. [17] While EDTA is used as a chelator, EDTA can also increase the number of soil microorganisms, but the number of nematodes was not found when given EDTA. The exact cause of the reduction in nematodes is unknown, but the use of a chelator (chelating agent) that can reduce the number of soil organisms needs to be avoided because it can disrupt the balance of the ecosystem.

2.4. Phytoremediation mechanism using chelating agents EDTA and Citric Acid

EDTA and Citric Acid as a chelating agents increase the mobilization of metals in the soil through plant root membranes and assist in metal translocation from roots to shoots [18]. The functions of chelating agents in enhancing phytoremediation are absorbing heavy metals by increasing their bioavailability, and chelating agents can bind heavy metals into HM-Chelator complexes. The chelating agent used must eliminate the factors that cause the limitation of phytoextraction of heavy metals from soil [11]. These factors are the low bioavailability of heavy metals in the soil and the low translocation of heavy metals from roots to plant shoots.

Chelating agents (chelators) form complexes in the root zone, thereby increasing the transport of heavy metals (HMs) to the aerial parts of plants [17]. The process of metal mobilization from the roots can be caused by:

a. Chelating agents stimulate root exudates (natural chelating compounds)
b. A chelating agent increases the transport and accumulation of the HM_s-Chelator complex in the rhizosphere
c. The process of plant transpiration affects the translocation of HM_s in plants.

The chelating agent forms a complex with heavy metals outside the plant, namely the HM-chelator complex. Then the HM_s-chelator complex enters the plant through the xylem. A chelating agent applied to soil contaminated with heavy metals will form an HM_s-Chelator complex, then the absorption process occurs through root absorption. The roots and water absorb the HMs-Chelator complex to the xylem by diffusion [18].

The HM-Chelator complex moves along the symplast through the plasmodesmata. It can also pass through the apoplast. When it reaches the Casparian strip, the HM-Chelator complex will enter the symplast flow. Further transport of solutes (including the HM-Chelator complex) from roots to shoots through plant transpiration. The chelator causes higher metal diffusion through the roots by increasing the metal concentration in the soil through metal desorption and lowering the metal diffusion coefficient in the complex form. Because it has a neutral charge complex, it will not be blocked by carboxyl groups or polysaccharides on rhizodermal cells [19]. In essence, chelators can cause the movement of HMs directly to the roots by binding to heavy metals to form complexes with HMs in the soil solution, then enter the plant through the roots and be translocated to the plant parts by the process of transpiration [19].

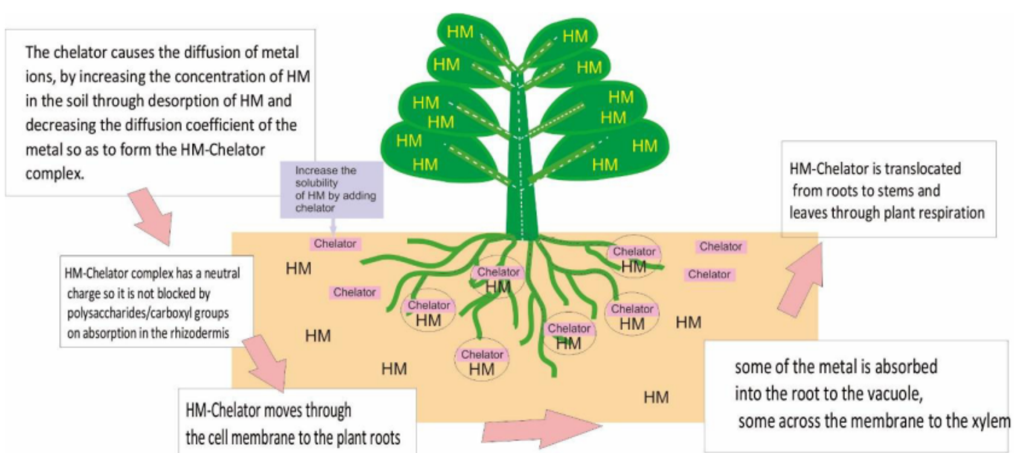

Figure 3. Phytoremediation mechanism using chelating agents.

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3. Advantages and disadvantages of Citric Acid and EDTA on the prospects of phytoremediation

There are several disadvantages of phytoremediation, one of which is that it takes longer than other remediation methods because the metal uptake ability depends on the number of heavy metals absorbed from the soil to the top of the plant. The slow growth of hyperaccumulator plants causes an increase in remediation time. Some heavy metals with low bioavailability also make it difficult for heavy metals to be absorbed by plants. Therefore, chelators are needed that help binds heavy metals to be absorbed by plants and increase phytoextraction. EDTA as a chelating agent proved to be effective in phytoextraction. Previous studies have shown that EDTA can bind various types of metals such as Pb, Cd, Cu, Cr, Zn and carry them to the top of the plant to be harvested. However, recent research has shown that EDTA poses a risk for groundwater contamination.

Based on a previous study, application of 2.7 mmol/kg EDTA was shown to increase phytoextraction, but at 5 months after application of EDTA to the soil, EDTA was still found in the soil pores. It proves that EDTA is difficult to degrade by soil microorganisms. In another study that described the effect of EDTA on phytoremediation, it was concluded that during EDTA application, there was an increase in heavy metal absorption to the shoots through transpiration, resulting in a reduction of heavy metals in the soil. However, after a few days, there was a significant increase in heavy metals in the soil[16]. It indicates that EDTA is effectively used as a metal chelator in phytoextraction but causes other environmental problems because the environment cannot degrade EDTA. However, the use of Citric Acid is less harmful to the environment. The environment quickly degrades Citric Acid, which is a natural substance. Citric Acid has a lower chelating ability than EDTA but does not cause further environmental problems [20]. Citric Acid will be degraded into organic matter needed by plants.

Table 2. Advantages and disadvantages of Citric Acid and EDTA on phytoremediation.

Chelator	Advantages	Disadvantages
Citric Acid	• Citric Acid is less harmful to the environment (biodegradable in soil) • Effective for phytostabilization • Citric Acid is cheap and easy to get.	• less effective on phytoextraction in some hyperaccumulator plants
EDTA	• EDTA is effective for phytoextraction on various heavy metals. • Cheap and easy to get	• The persistence of these chelating agents in the environment creates additional and unforeseen problems (risk of groundwater contamination)

The use of Citric Acid and EDTA each has advantages and disadvantages. Citric Acid is easy to obtain and relatively cheap, and has phytoextraction and phytostabilization, but it is not as good as EDTA in phytoextraction. In comparison, EDTA is an excellent metal chelator in phytoextraction but poses a risk of groundwater contamination because of its persistence in the environment or nonbiodegradable.

In the prospect of phytoremediation using chelating agents, Citric Acid can be recommended for remediation of polluted soils that have high permeability, on the other hand, EDTA can be used on low permeability soils with small doses because of concerns about the leaching of EDTA to groundwater which is dangerous for environmental. In future research, it is hoped that there will be potent chelating agents, both synthetic or organic, that can increase the ability of phytoextraction and are biodegradable so that they are less harmful to the environment.

4. Conclusion

From the results of the review, EDTA and Citric Acid are effective in increasing phytoextraction and phytostabilization. Furthermore, on the effect of Citric Acid on soil physiology, Citric Acid can decrease

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hydraulic conductivity due to peptizing as a barrier to porous soils. Citric Acid also promotes root growth, causing compaction of the surrounding soil, increasing soil mass density, while EDTA does not change soil physiology.

In the effect of Citric Acid on soil microbiology, there was an increase in bacterial biomass in polluted soil compared to control soil. Citric Acid can stimulate plants to release exudate and stimulate root growth, making it the best place for microorganisms to grow. Meanwhile, with the addition of EDTA, soil microbiological biomass increased, but nematodes decreased significantly.

In the mechanism of EDTA and Citric Acid in Phytoremediation, chelators cause the movement of heavy metals (HM) directly to the roots by binding heavy metals to form complexes with HM in soil solution, then the HM and chelator bonds enter the plant through the roots, and are translocated to plant parts through transpiration process.

EDTA has a high chelating ability for phytoextraction but is difficult to degrade by the environment, so its application in the field can potentially contaminate groundwater due to EDTA leaching. While Citric Acid is best for phytostabilization, it is also suitable for phytoextraction. The other advantages of Citric Acid are less harmful to the environment (biodegradable).

Citric Acid can be recommended for remediation because it does not cause further environmental problems in the prospect of phytoremediation using chelating agents. In future research, it is hoped that there will be potent chelating agents, both synthetic or organic, that can increase the ability of phytoextraction and are biodegradable so that they are less harmful to the environment.

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