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Effect of chelating agents on heavy metal extraction from contaminated soils

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Abstract- In the present study shacking and incubation experiments were carried out in order to evaluate the changes in heavy metal solubility in the studied soils by addition of different concentration of Ethylene dinitrilo tetra acetic acid (EDTA), Diethylene triamine penta acetic acid (DTPA), and Nitrilo tri acetic acid (NTA). The effects of EDTA, DTPA and NTA application on solubility of Copper (Cu), Lead(Pb), Cadmium(Cd) and Chromium(Cr) in soil were evaluated. In shacking experiment, maximum Cu, Pb, Cd and Cr were solubilized by DTPA extractant. It was found that with increasing chelating agent doses metals availability was increased and 5.0 mM doses of EDTA, DTPA and NTA was noticed the best optimum dose for further experiments. For shacking time significant results were obtained at 120 hours by applying EDTA and NTA where as DTPA behaved well at 24 hours. In incubation experiments more Cu and Cd were extracted by DTPA 6.65 and 6.67 ppm respectively. EDTA was proved good extracting solution for Pb which has solubilized maximum concentration of Pb (22.816 ppm). Maximum concentration of Cr (1.335 ppm) was solubilized by NTA as compared to EDTA and DTPA. For incubation experiment day 20-30 were more suitable for solubilzation of metals. Chelats has potential for the remediation of heavy metal-contaminated soils either as on-site soil washing agents or for in situ remediation. These findings can be used to develop a predictive tool for the target metals contaminants and for assessing chelation remediation efficiency based on chelates dose and contact time test results.
Index term- Chelating agents, contaminated soils, heavy metals, extraction, and remediation

1 INTRODUCTION

UNCONTROLLED waste disposal and poor practices by humans leads to soil contamination. Due to atmospheric deposition, non-biodegradability and leaching ability heavy metals are those pollutants which are of particular concerns. In Pakistan different industries like iron foundries, leather industries, chemical , fertilizer and textile mills are discharging their heavy metal containing effluents into the agricultural lands and water bodies and this water is being used for irrigation, polluting the soil and the food chain [1]. Therefore, in the plough-layer of soils, in vegetation, animals, lakes, rivers, and even in the oceanic regions and also in human beings the level of a variety of elements have substantially increased with the passage of time. Soil is source and sink of metals. The total and bioavailable concentrations of heavy metals in soils are of great importance with regard to human toxicology.

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As heavy metals are not biodegradable and therefore they remain in ecological systems and in the food chain indefinitely, which ultimately expose the top-level predators to very high level of pollution [2]. Soil remediation is a challenge for scientist and regulatory authorities. This can be done by using physical, chemical and biological techniques but they do not offer satisfactory solution for many sites especially for agricultural lands.
In biological treatments phytoremediation is a cost effective technique because it is being accepted by the public due to aesthetic sense. It is also more economic and environmental friendly. It involves the usage of green plants for removal of pollutants or alters them to less toxic form [3]. Phytoremediation can be enhanced or assisted by using chemical and biological agents which leads to more accumulation of metals in plants [4].
A chemical agent may be a chelate which is composed of a metal ion and a chelating agent. It can form several bonds to a single metal ion. It can also forms strong, water soluble metal complexes with di- and trivalent cations. Chelating agents have been used for supplying micronutrients to the plants from more than 50 years. [5]. Chelates maintain their bioavailability for plants by preventing precipitation and sorption of metals [6].
The addition of chelating agents to the soil can also bring metals into solution through desorption of sorbed species, dissolution of Fe and Mn oxides, and dissolution of precipitated compounds [7]. These complexes can greatly alter the reactivity of the metal ion. They can alter the oxidation – reduction properties of transition-metal ions, such as iron (Fe) and manganese (Mn), and therefore increase or decrease the reactivity of these systems. To achieve the productive phytoextraction phytoavailability of the metals is a very important factor. The availability of metals limits the efficiency of Phytoextraction [8].
Chelating agent like EDTA has ability to form water soluble complexes by increasing uptake of metals desorbing from the solid phase of soil [9]. Metal is basically portioned in two phases: reversible and irreversible. Chelats tries to absorb metals from reversible and then from irreversible phase [10]. This basic principle is used to test the chelating agent, that how well chelate can free the metals from the bounded phase. If they are unlocked from irreversible phase their efficiency is more. Recently it is explored that EDDS and NTA have their biodegradability as opposed to EDTA so that is being employed for metals extraction [11].
Shaking time is an important factor in governing the heavy metals solubilization in soil matrix. The ability of chelating

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agent to sustain heavy metals in a soluble form is affected by the stability of the complex. As shown [12], a steady state condition between EDTA and Pb was not achieved within one day in Pb contaminated soil. So by studying the time factor we can determine the availability and persistence of the chelant in the soil matrix.

Incubation time should also be taken in to account when evaluating the metals solubilization efficiency of the chelants. Chaney [13] reported that oxidation of metal ion and formation of metal-chelate complex may take different period of time for different metals. So, incubation time should not be ignored while evaluating other environmental impacts. In addition to this incubation period helps in determining the biodegradation period of chelants. The goal of present study is to evaluate the chelating ability of chemical and biological agents to solubilize heavy metals from contaminated soil, which as a result will later on assist the plants in the uptake of heavy metals from contaminated soils and rectify it. The present research work was conducted to assess the efficiency of different chelating agents like EDTA, DTPA and NTA and optimal dose/ concentration of chelating agent. Maximum solubilization of the meta ions (Cu , Pb, Cd and Cr) at suitable shacking intervals and incubation period was also analyzed.

2. Methodology

2.1 Selection of the contaminated soils

Using the criteria for low to moderate level of heavy metals (Pb, Cd, Cr and Cu) contamination, contaminated soil was selected from the Gujranwala site. Bulk surface samples of a heavy metals polluted arable soils; i.e. Soil-Gujranwala (loamy, mixed, hyperthermic Udic Haplustalf) was collected from peri-urban areas of Gujranwala (N 32o-06.262; E 74o-10.236) for laboratory and pot experiments.

Note: This soil was under untreated municipal/industrial effluent irrigation for the last more than 20 years. This was formed from alluvial sediments. The parent material is of mixed nature and have been derived a wide range of rocks.

2.2 Soil preparation

Contaminated soil was collected from 0-30 cm surface layer of the peri urban an agricultural soil of Gujranwala and used in laboratory experiments. The soil was air-dried at room temperature, homogenized, sieved through a 2-mm sieve and characterized as follows. The sand, clay and silt fractions of the samples were determined by the hydrometer methods and the organic matter content was determined by the Walkley-Black method [14. The pH and electrical conductivity in a 1:2 soil: water ratio (and calcium carbonate [15].

The initial total Cu , Cd, Cr and Pb content of the soil, as determined by the acid digestion method [16]. Analysis of Cu, Cd, Cr and Pb analysis in the filtrate was performed by flame FAAS (Perkin-Elmer AAnalyser 800). Standards for the FAAS calibration were prepared in the extraction solution by the addition of appropriate quantities of Cu, Cr, Cd and Pb respectively. The soil properties are listed in Table 1.

Table. 1. Characteristics of soils used in shacking and incubation experiments.

Parameter	Gujranwala Soil (Udic Haplustalf)
pH (1:2)	7.83
EC (dSm-1)	0.47
O.M. (%)	1.36
CaCO3 (%)	1.9
CEC (mq/100 g soil)	10.29
Particle size distribution (%)	
Textural	Sandy Clay Loam
Total heavy metal contents (mg kg-1)	
Lead	270
Cadmium	7.8
Chromium	331.8
Copper	331.9

2.3 Shaking Experiment (Efficiency of different chelating agents)

A replicated laboratory experiments were done for the selection of most suitable chelate materials, concentration and shacking time to enhance the solubility of heavy metals in polluted soils. Experimental soil was treated with different chelating agents, i.e., EDTA, DTPA, and NTA, applied @ 0, 1.25, 2.5 and 5 mM kg-1 soil.

About 15 g of soil was weighed into 125 ml conical flasks. The flasks were pre-washed with dilute HNO3 to eliminate any adsorbed metals. The soil was amended with chelates solution (1:5) either, DTPA, EDTA, NTA, or deionizer water (control). The sample flask was covered with parafilm and then shaken at room temperature for 6, 24, 48, and 120 hours at 125rpm and supernatants were filtered subsequently through a filter paper (Whatman No. 42 filter paper). The filtrate was stored in a clean plastic bottle for later metal analysis. Analysis for metal (Pb, Cd, Cr, and Cu) concentrations was done using Atomic Absorption Spectroscopy with Graphite Furnace. Each treatment was performed in triplicates.

2.4 Incubation Experiment

Incubation experiment was carried out in order to evaluate the changes in heavy metal solubility in the studied soils by addition of different concentration of EDTA, DTPA, and NTA. Contaminated agricultural soil collected from Gujranwala areas was selected for this incubation experiment in the period of 0.25, 1, 2, 5, 7, 10, 20 and 30 days, treated with different concentration of chelate (0, 1.25, 2.5 and 5.0 mM kg-1 soil) and was carried out under controled conditions. Twenty grams (20g) of air-dried soil was placed into acid-cleaned 125-ml polyethylene bottles. An amount of 3.4 ml (~ 60% water field capacity) of the EDTA, DTPA and NTA solution (0, 1.25, 2.5 and 5.0 mM/l) was added to the soil. The control variant was treated with

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3.4 ml of deionised water. Samples were extracted with 50 ml of deionised water for 1 h on an end-over-end shaker at 30 rpm and filterd. Water-soluble contents of heavy metals (Pb, Cd, Cr, and Cu) in solutions were analyzed by using atomic absorption spectroscopy with graphite furnace.

3 Results and Discussion

In the present study the influence of the addition of chelating agents EDTA, DTPA and NTA, and their relationship with the availability of Cu, Pb, Cd and Cr in contaminated soils, was assessed under shaking and incubation experiments.

3.1 Shacking Experiments

3.1.1 Effect of shacking time and concentration on metals solubilization

EDTA: The evolution of soluble heavy metals after the addition of EDTA is given in Figure 1. EDTA was successful at mobilizing the target heavy metals (Cu, Pb, Cd, Cr) from the soil. It is observed from figure 1(a) that with increasing reaction time the solubilisation of Cu slightly increased in first 6h at 0, 2.5 and 5.0mM concentration of EDTA where as no significant difference was observed in 6, 24 and 48h when EDTA concentration was kept 1.25 mM. With increasing shacking time all the three applied concentrations of EDTA showed slight increase in Cu availability but soil without EDTA treatment depicted the reduction in Cu concentration at 120h. Maximum Cu solubility was observed at 5.0 mM EDTA which concluded that EDTA application enhanced the Cu availability.

Figure 1(b) shows that EDTA concentrations 1.25 and 5.0 mM slightly increased the Pb solubility from 6 to 24h contact time but decreased from 24 to 48h and remained same from 24 to 120h shacking time. However Pb availability at 5.0 mM EDTA decreased with increase in shacking time. Similar behavior was also observed by the control soil sample. Overall it was noticed that increased concentration of EDTA also increased the Pb solubility.

It is evident from figure 1(c) that initially EDTA application showed good results and maximum solubilization was observed by 5 mM concentration of EDTA. But later on due to increase in shacking time from 6 to 24h, increase in Cd concentration was observed in samples with EDTA application of 0, 1.25, and 2.5 mM. At 48h all samples showed downward curve by following similar pattern. However till 120 h shacking times a little change was observed. Overall 5 mM EDTA concentration showed good results at 6h and 0, 1.25 and 2.5 mM behaved well at 24h shacking time.

Chromium solubilization was observed in figure 1(d). These results indicate that from 6 to 24h shacking time Cr availability was enhanced whereas at 48h a decrease was observed except 1.25 mM EDTA concentration. After that solubilization was moderately raised for all the applied doses of EDTA including control.

The application of EDTA to the contaminated soil increased the levels of solubilized Cu, Pb, Cd and Cr as has been reported in previous studies [17]. According to Chen et al [18] the addition of EDTA to contaminated soil increases the soluble or exchangeable fraction of heavy metals in the soil solution making them available for uptake by plants. In our study, significant amount of the soluble fraction of Cu, Pb, Cd and Cr can be observed in the soil solution for several hours following the application of EDTA. This shows that the solubilizing effect of EDTA did not decrease with time which could be due to its high environmental persistence [19].

The extraction of heavy metals form contaminated soils by different suitable chelators and soil characteristics are major tasks for remediation. The first step is to identify the selectivity and efficiency of the chelating agents. Examinations have been carried out to review the strength of EDTA in solubilizing Pb from the contaminated soil [20]. and EDTA performed well in recovering Pb from the soil. In another research the effect of pH was studied on Pb contaminated soil from battery reclamation site. According to the results the release of Pb was greater at elevated concentrations of EDTA, which is in accordance to the results of present study (figure 1 & 4) furthermore acidic pH favored more Pb recovery because lower pH favors leaching of metals [21].

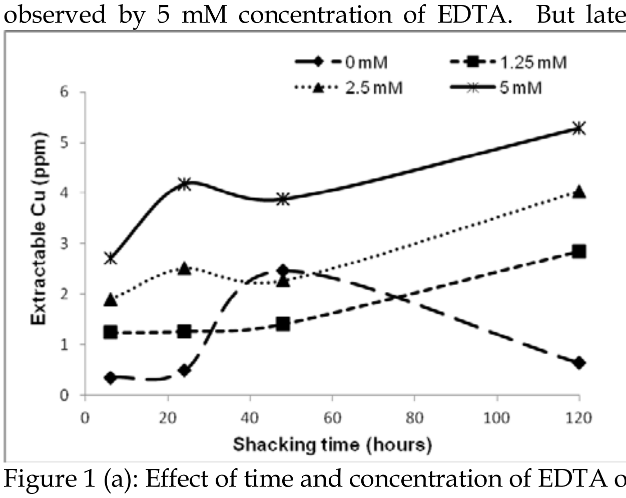

Figure 1 (a): Effect of time and concentration of EDTA on Cu solubilisation.

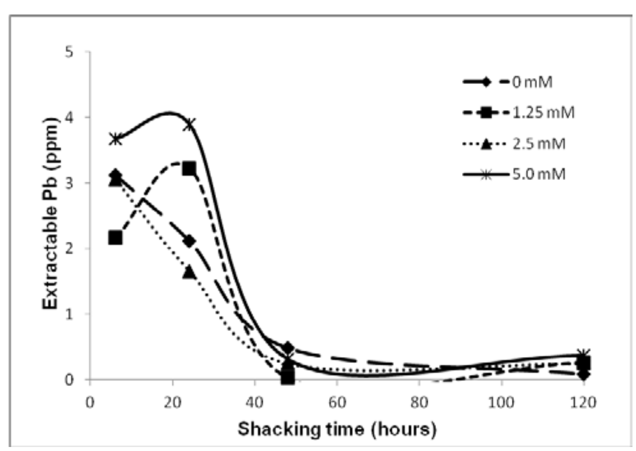

Figure 1 (b): Effect of time and concentration of EDTA on Pb solubilisation.

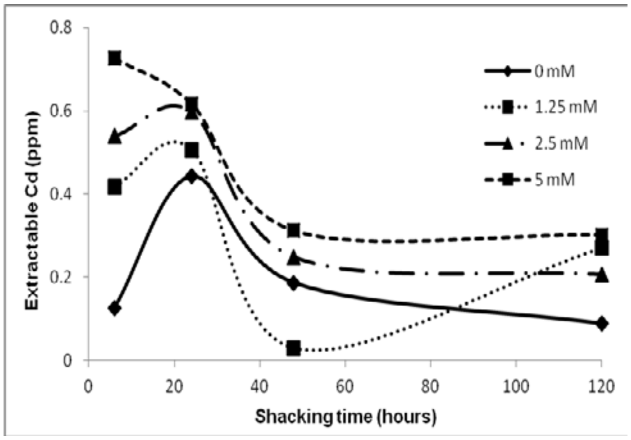

Figure 1 (c): Effect of time and concentration of EDTA on Cd solubilisation.

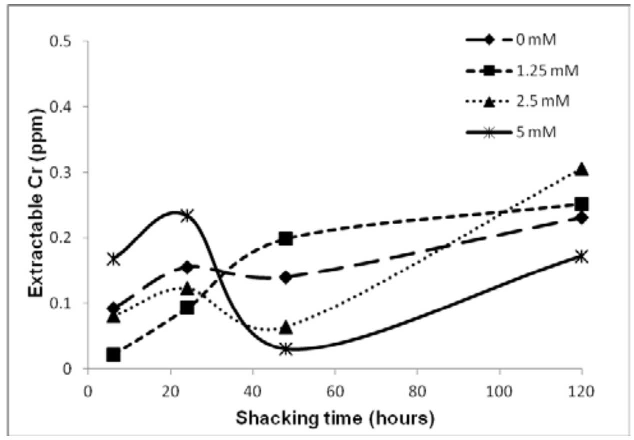

Figure 1 (d): Effect of time and concentration of EDTA on Cr solubilisation.

DTPA: The solubility of heavy metals after the addition of DTPA is evaluated and given in Figure 2. DTPA was successful at mobilizing the target heavy metals (Cu, Pb, Cd, Cr) from the agricultural soil.

It is noticed from figure 2(a) that different DTPA concentrations (0, 1.25, 2.5 and 5 mM) depicted difference in Cu solubilization but exhibited almost same pattern. Cu concentration was increased slightly from 6 to 24h then slightly reduced and then became same at 120h (47.9, 50.8 and 51.9 ppm for 1.25, 2.5 and 5 mM DTPA respectively). And these results also showed increase in Cu availability due to the amendment of DTPA as control soil samples.

It is observed that with increase in DTPA concentration Pb availability was also increased (figure 2(b). Maximum Pb availability was made successful by 5 mM DTPA concentration. However shacking time had not shown any drastic change in Pb solubilisation. Figure 2(c) exhibit the chelation of Cd. 5.0mM DTPA concentration showed highest solubilisation of Cd as compared to the 0, 1.25 and 2.5 mM DTPA. Less effect of shacking time was observed, only 5 and 2.5 mM DTPA showed variation in solubilisation graphs at 48h otherwise it almost remained constant.

Cr solubilisation is presented in figure 2(d). Maximum solubilisation was given by DTPA concentration of 5.0 mM at 6h while it became same at 24h except 1.25 mM further graphs of four levels of DTPA united at same point at 48h but at the end of experiment, 2.5 and 5.0 mM concentration of DTPA depicted higher solubilisation than 0 and 1.25 mM. So these results showed that by increase in shaking time Cr availability also increased.

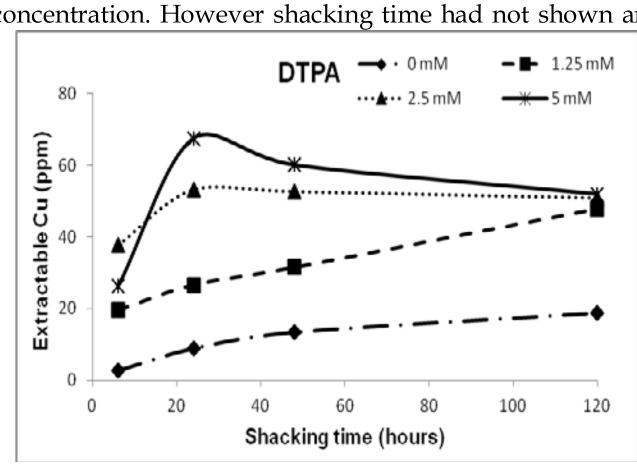

Figure 2 (a): Effect of time and concentration of DTPA on Cu solubilisation.

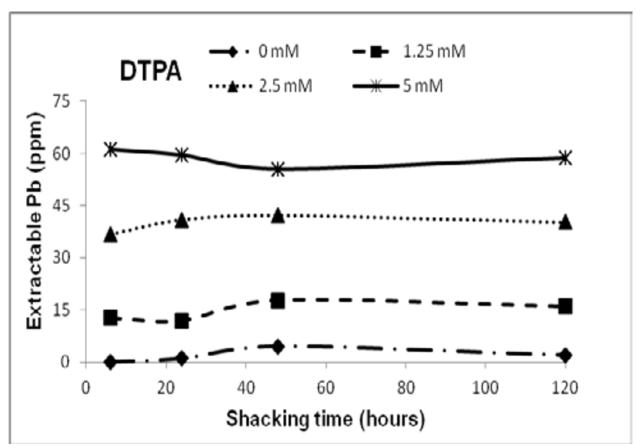

Figure 2 (b): Effect of time and concentration of DTPA on Pb solubilisation.

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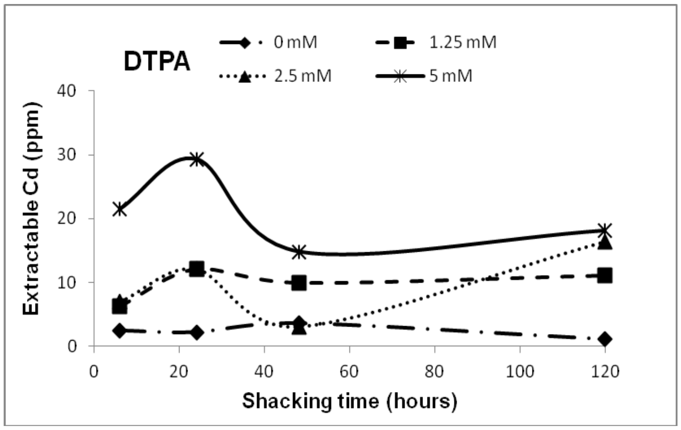

Figure 2 (c): Effect of time and concentration of DTPA on Cd solubilisation.

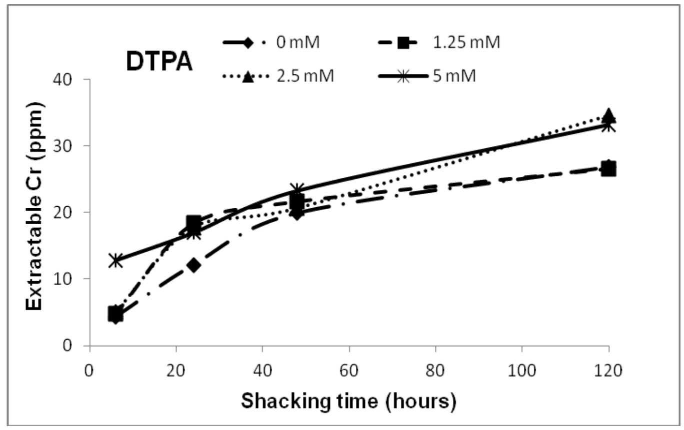

Figure 2 (d): Effect of time and concentration of DTPA on Cr solubilisation.

NTA: The solubility of Cu after the addition of NTA was evaluated and presented in figure 3(a). It is noticed that with increase in NTA concentration Cu availability increased. Control soil samples showed better solubilisation at 6 h but at 24h it was reduced and remained almost constant till 120h. Less effect was observed for shacking time for samples amended wit NTA, however control showed some variations in the start.

Figure 3(b) depicted that Pb availability was more at 6h when soil samples were amended with 2.5 and 5.0 mM of NTA than controled and 1.25 mM. An opposite behavior was observed at 24h. After 48h shacking Pb solubilization became same for all the applied concentrations of NTA. However at 120h Pb solubilisation was increased in all treated samples as well as in the control samples.

The results presented in figure 3(c) show that Cd availability increased by increasing shacking time from 6h to 120h. Maximum Cd solubilisation was observed in soil samples treated with 5 mM concentration of NTA. So both chelate concentration and shacking time have positive effects on Cd availability.

Figure 3(d) showed that similar effects on the availability of Cr were observed as for Cd. However little bit fluctuation at 24h by 1.25mM of NTA concentration was noticed.

As chelating agents are known to be effective extractant of heavy metals from contaminated soils therefore, this study was aim to compare the different concentrations and treatment times of EDTA, DTPA and NTA to test the extraction of Cu, Pb, Cd and Cr to determine the best dose, shacking and incubation time for phytoremediation experiments.

A research was performed by Peters and Shem [22] who involved the extraction of Pb by EDTA and NTA from a soil containing 70% silt and clay. EDTA removed 68.7% of Pb by taking less time, whereas NTA removed 19.1% Pb and it took longer time as compare to EDTA. It was observed that soil comprising more than 70% sand resulted in 85% solubilized Pb probably because soil having more clay will bind to the metal, but sand in soil will not have adsorptive capacity like clay and therefore more Pb would be chelated. This study determined the clay content (24%) that was slightly greater than sand (14%) and silt to be 61%, so these statistics can explain the sorption of metal with soil and its solubilisation behavior upon addition of chelating agents. A more provoking thought however here is the exploitation of the relation between sorption of metals and clay content of soil.

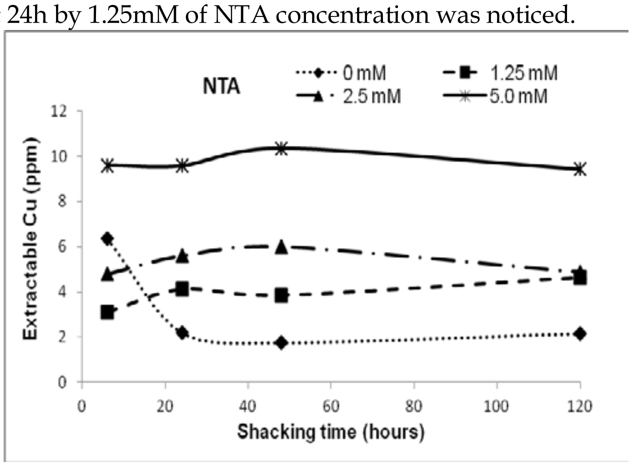

Figure 3 (a): Effect of time and concentration of NTA on Cu solubilisation.

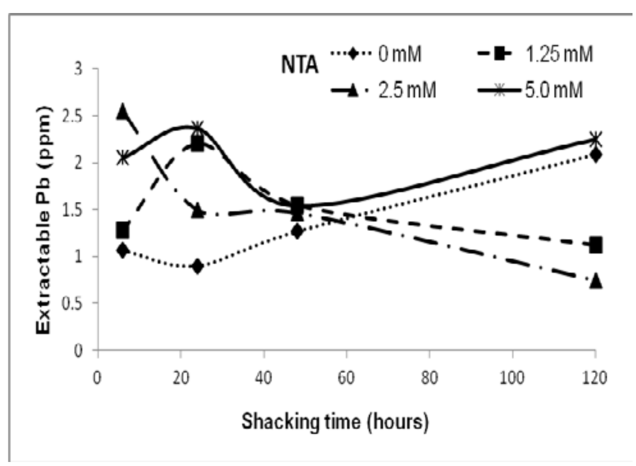

Figure 3 (b): Effect of time and concentration of NTA on Pb solubilisation.

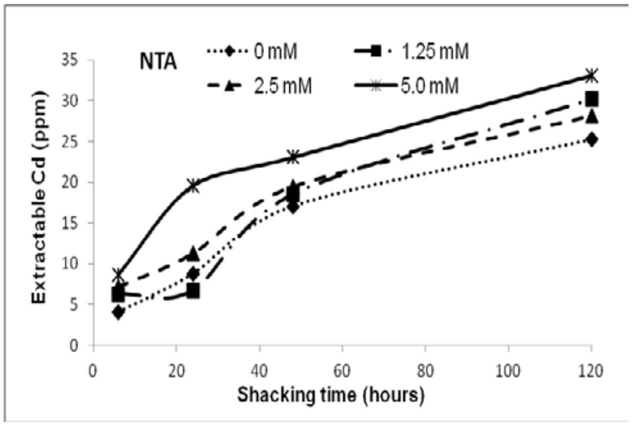

Figure 3 (c): Effect of time and concentration of NTA on Cd solubilisation.

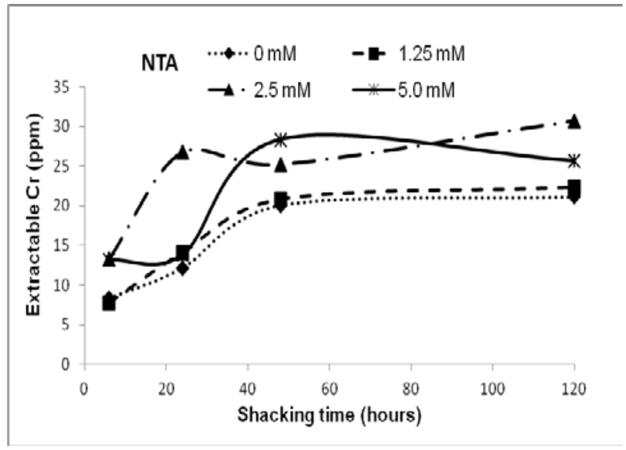

Figure 3 (d): Effect of time and concentration of NTA on Cr solubilisation.

3.2 Incubation experiments

3.2 .1 Effect of incubation time and EDTA concentrations on metals extraction

In general, the chelating agents were efficient in the solubilisation of heavy metals from the contaminated soil. The extent of metal solubilisation varied according to soil characteristics, chelating agent and the specific metal.

EDTA: This experiment was conducted to check the effect of EDTA concentration (0, 12.5, 2.5 and 5.0 mM) and incubation days (0.25, 1, 2, 5, 10, 20 and 30) on solubilisation of Cu, Pb, Cd and Cr. EDTA extractable heavy metals contents increased during the incubation and mostly reached its maximum value. Figure 4 (a) exhibits that Cu availability increased from 0.25 to 20 days and it showed little decline on day 30. Maximum Cu availability was noticed on day 5 (2.84 ppm).

For the solubilised amounts of Pb an increase on the extracted amount can be related to an increase in the applied rates (Figure 4b). Maximum Pb solubilization by EDTA was observed at day 10 and 2.5 mM applied concentration. On day 10 and 20 better results were observed. But on day 30 a slight decline was noticed as compared to day 10 and 20.

By looking at figure 4(c), It is evident that by increasing incubation days Cd availability also increased. EDTA dose 5.0 mM showed best results among the all applied doses of EDTA. It is observed from the given results that effect of 2.5 mM dose of EDTA on activating the Cd availability also showed behavior close to the dose 5.0 mM.

Maximum Cr availability was obtained at day 20 and 1.25 mM EDTA concentration. Control soil samples also showed Cr solubilisation from day 2 to 30. There was less variability in the effect of different concentrations of EDTA in extraction of Cr. However Cr extractability was increased by increasing incubation days.

It is concluded that EDTA can extract heavy metals even at low concentration. It is proved as good extractant for Pb. The order of availability of metals by forming metal-chelate complex was Pb> Cu> Cr and Cd. EDTA performed best from day 1 to 30 for Cu, 2 to 30 for Cd, 5 to 30 for Pb and 10 to 30 for Cr.

A consistency was observed, the data shows that effective chelation was observed on day 20 and 30, in most cases, followed by a decrease in solubilization. It was highlighted in an investigation that there are two steps involved in the mobilization of metals: a fast step and a slow step. In the former situation the accessible metals, which are in exchangeable and slightly adsorbed form, are solubilized first. In this specific study Cu was more solubilized in the fast step. The mechanism followed in the slow step correlates with chelation of metals that are somewhat less available and mobile, or in other words metals at this stage are bound to oxides [23]. This would serve as a valid explanation as to why on day 20 and 30 of treatment, rather than on other days, most active chelation took place. Because metals were being solubilized from the storage of accessible metals for the limited time period, after which the metals could no longer be available.

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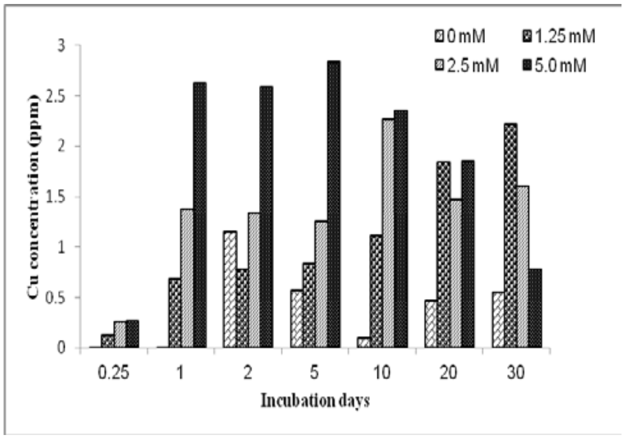

Figure 4 (a): Solubilized Cu at differentiate concentrations and incubation time.

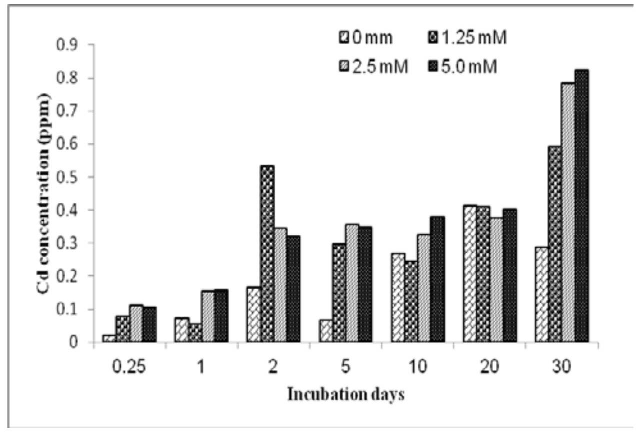

Figure 4 (c): Solubilized Cd at different EDTA concentrations and incubation time.

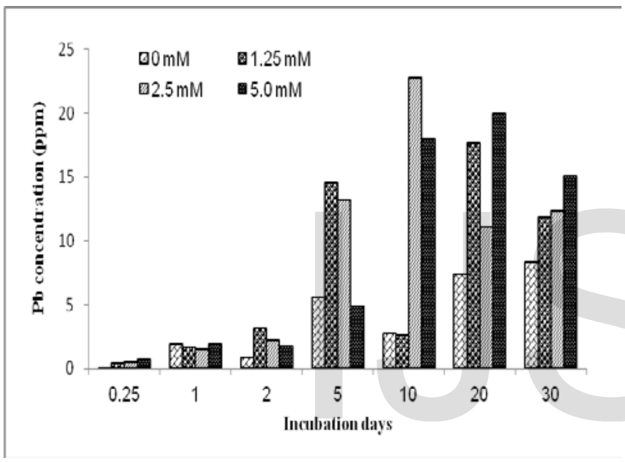

Figure 4 (b): Solubilized BP at different EDTA mmconcentrations and incubation time.

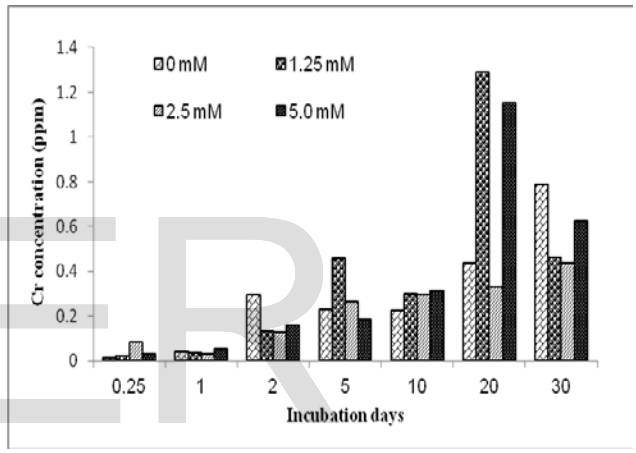

Figure 4 (d): Solubilized Cr at different EDTA concentrations and incubation time.

DTPA: The results of the Cu concentration as affected by incubation interval and addition of DTPA are presented in figure 5(a). The concentration of Cu increased with the passage of time and the magnitude of increase was different when amended with different concentrations of DTPA (0, 1.25, 2.5 and 5.0 mM). Significantly higher concentration of Cu was noted in the soil treated with 2.5 mM and the lowest was noted in untreated soil. It was noted that higher values of Cu were recorded in soil on day 20 followed by day 30, 10, 5, 2, 1 and 0.25.

Figure 5(b) exhibits that Pb solubilisation was initially increased with incubation time and then a decline was observed in graph after day 10. Maximum availability of Pb (9.64 ppm) was noticed on day 10 and 1.25 mM DTPA dose. On day 0.25 and 1 Pb availability was almost absent except 2.5 and 5.0 mM doses of DTPA.

Mobilization of Cd by the addition of DTPA was maximum at 5.0 mM concentration on day 20 (figure 5 (c). It is noticed that by increasing incubation period solubilisation was increased and a peak was noticed at day 20 and after that a slight decline was noticed on day 30. On day 0.25 and 1 there was no significance difference in concentration of solubilized Cd by different applied doses of DTPA. However at day 2 and 5 solubilisation was increased by raising concentration of DTPA but on day 10 a contrasting results were shown. Instead of 5.0 mM, 2.5 mM dose of DTPA showed better results. However DTPA concentration 5.0 mM behaved well on day 20 and 30.

Figure 5(d) depicted the availability of Cr in the presence of different concentrations of DTPA on different incubation time period. In this case control samples also showed good results.

A sharp increased was noticed from day 1 and which was maintained till the end of experiment (day 30). Maximum Cr availability was noticed on day 30. All the applied doses of DTPA showed effective solubilisation of Cr. Maximum concentration of Cr was observed 0.312 and 0.302 in soil samples treated with 2.5 and 1.25 mM DTPA respectively.

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The data indicates that less time is required in Cr solubilisation.

The result of present study indicates that trend of water soluble Cd increases with DTPA application in soil (observed validate earlier studies where metal concentration in leachate after chelator addition was clearly related to the rate of chelator applied. After 20 and 30 days of DTPA application, a strong effect of DTPA on Cu, Pb, Cd and Cr solubility was observed which show the persistency of DTPA in soil [24]. Copper, lead, cadmium and chromium recovery in our study was more at 5.0 mM DTPA kg_1 soil than in the control with no added DTPA. Outcome of chelator application in terms of manifold increase in solubility of Cd in soil leachate water is well known [25].

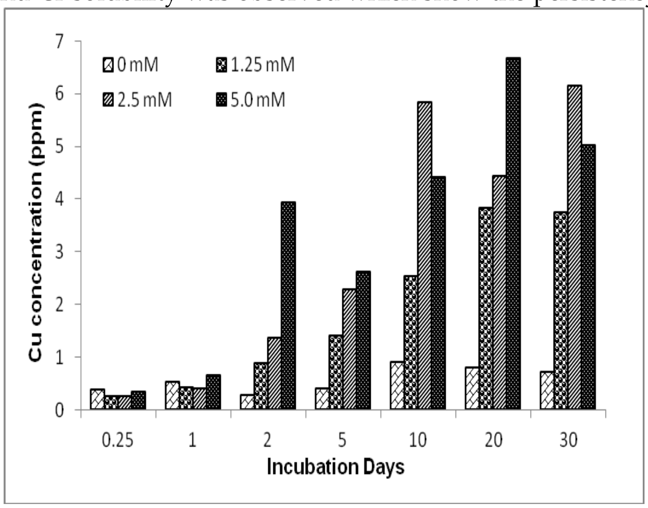

Figure 5 (a): Solubilized Cu at different DTPA concentrations and incubation time.

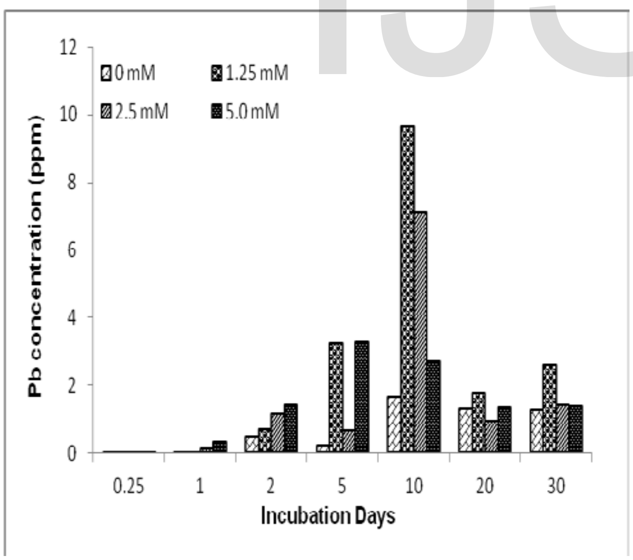

Figure 5 (b): Solubilized Pb at different DTPA concentrations and incubation time.

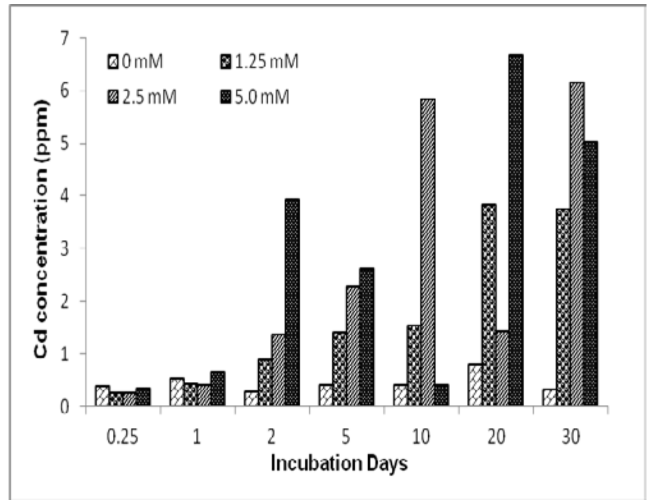

Figure 5 (c): Solubilized Cd at different DTPA concentrations and incubation time.

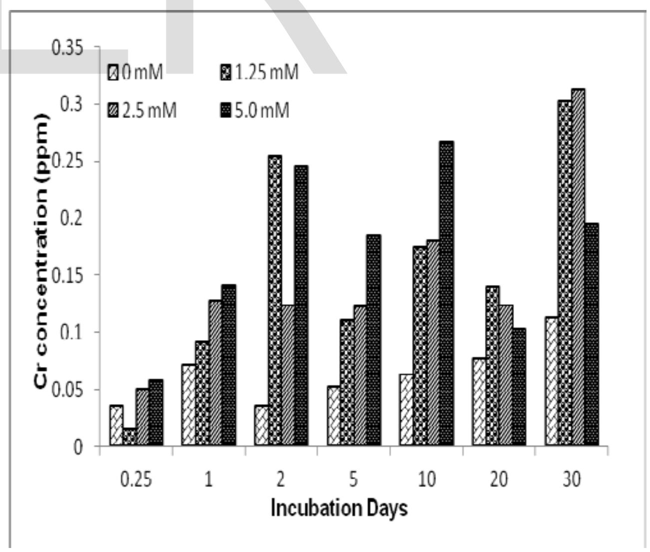

Figure 5 (d): Solubilized Cr at different DTPA concentrations and incubation time.

NTA: NTA was applied to promote the availability of soil Pb as shown in the figure 6(a). It is noticed from the results that NTA enhanced the Cu availability and NTA activity was increased with increasing incubation time from day 0.25 to 5. On day 10 and 20 NTA showed slightly low activity as compared to the previous days. However again a sharp increment was observed on day 30. 5.0 mM NTA application gave good result as compared to other doses 1.25 and 2.5 mM and control. So we can conclude that 0.5 mM NTA dose and incubation period of 30 days is best for Cu solubilization.

Figure 6(b) indicates that NTA chelation was less in the initial period of incubation (from 0.25 to 10 days) but it increased suddenly on day 20 and 30. Maximum solubilisation of Pb was obtained 20.04 and 19.53 ppm on day 30 and 20 and soil samples were treated with 1.25 and 5.0 mM NTA doses. Overall it was seen that NTA concentration variation exhibited less effect on the chelating ability. However time variation showed slow response in the starting of experiment but a sharp increase was noticed till day 20.

Figure 6(c) demonstrates the Cd solubilisation with different doses of NTA and incubation period. Maximum availability of Cd was observed on day 5 and 2.5 mM NTA applied dose. These results show slightly different behavior than other metals. The chelation activity was on its peak in the middle of experiment (on day 5) as compared to the start and end of the experiment.

Cr solubilisation with different NTA doses against incubation time is shown in the figure 6(d). NTA activity was better from the initial period of the experiment (day 0.25) and increased by increasing experimental time. Maximum Cr concentration was observed at day 30 in soil treated with 1.25 mM NTA. By looking on the behavior of applied doses of NTA it was seen that there was less difference in solubilisation of Cr and untreated soil samples also showed better results. Overall incubation period of 30 days and NTA concentration of 1.25 mM was best option for the further experiments.

The potential use of NTA in extraction of Cu, Pb, Cd and Cr was studied by applying 0, 1.25, 2.5 and 5.0 mM concentration for different time intervals to contaminated soils and measuring the solubilized levels of heavy metals over a period of and 30 days. Addition of NTA to contaminated soils increases the soluble or exchangeable fraction of heavy metals in the soil solution making them available for uptake by plants. In our study, significant amounts of the soluble fraction of Cu, Pb, Cd and Cr can be observed in the soil solution several days following the application of NTA. This shows that the solubilizing effect of NTA not decreases with time which can be due to high environmental persistence of NTA (Lombi et al., 2001).

This work evaluates the effects of EDTA, DTPA and NTA application on Cu, Pb, Cd and Cr solubility in metals contaminated calcareous soil. For phytoextraction of these metal ions the use of EDTA, DTPA and NTA as synthetic chelators seems suitable. The operational cost of chelant-enhanced phytoremediation is much lower than the soil washing operation. In combination with the possible recovery of extracted metals, this technology can be more promising in the future. However, the potential leaching of metals into surrounding environments is the most important concern in this process. It is therefore essential to optimize this technology before it can be safely adopted in field applications.

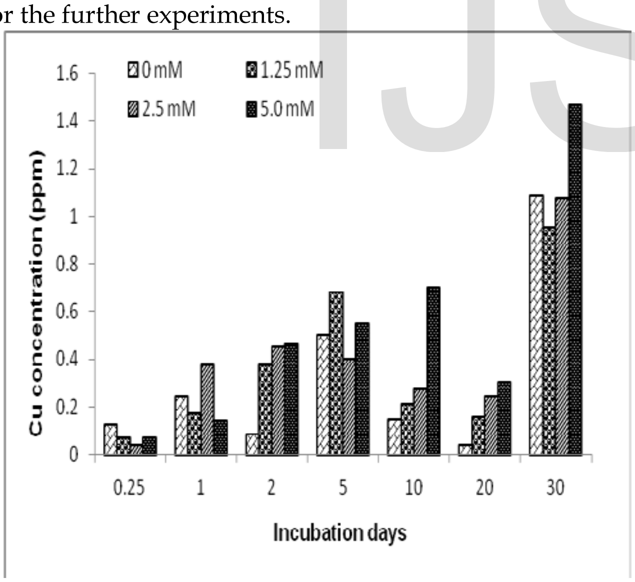

Figure 6 (a): Solubilized Cr at different NTA concentrations and incubation time.

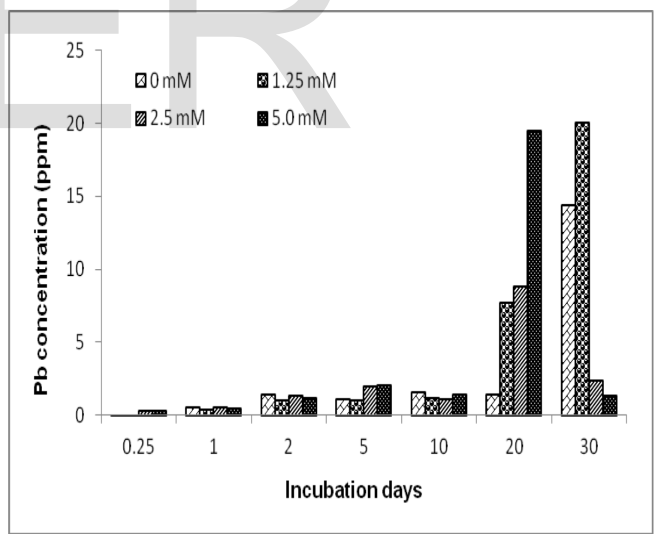

Figure 6 (b): Solubilized Pb at different NTA concentrations and incubation time.

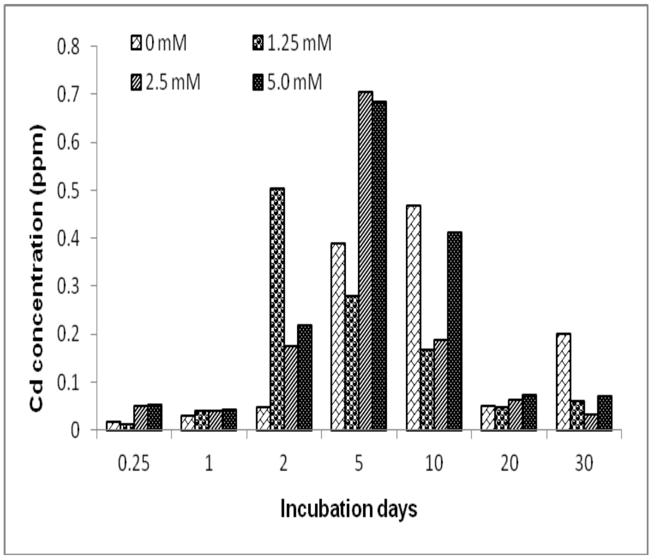

Figure 6 (c): Solubilized Cd at different DTPA concentrations and incubation time.

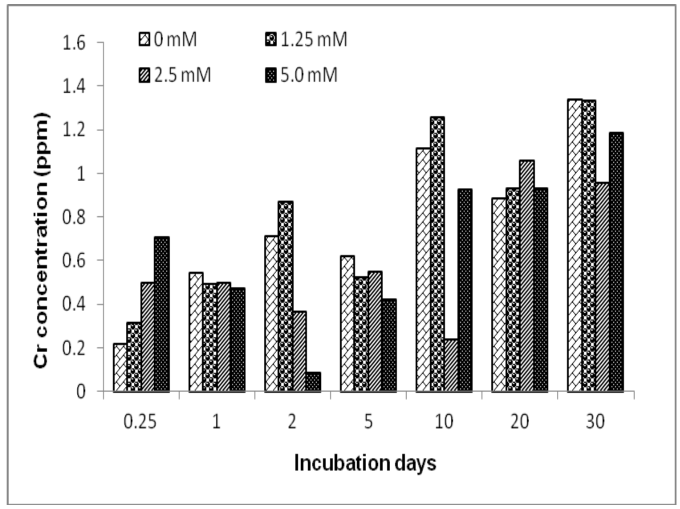

Figure 6 (d): Solubilized Cr at different NTA concentrations and incubation time.

4. Conclusion

In shacking experiment, maximum Cu, Pb, Cd and Cr were solubilized by DTPA extractant. DTPA is therefore good for solubilization and in turn remediation of contaminated soils. It was concluded that by increasing chelating agent doses metals availability was increased and 5.0 mM doses of EDTA, DTPA and NTA was noticed the best optimum dose for further experiments. For shacking time significant results were achieved in 120 hours by applying EDTA and NTA where as DTPA behaved well in 24 hours. In incubation experiments more quantity of Cu and Cd was extracted by DTPA 6.65 and 6.67 ppm respectively. Whereas EDTA was proved good extractant for Pb which has solubilized maximum concentration of Pb (22.816 ppm).Maximum concentration of Cr (1.335 ppm) was solubilized by NTA as compared to EDTA and DTPA. For incubation experiment day 30 and 20 were more suitable for solubilzation of metals.

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