Chelating Impact Assessment of Biological and Chemical Chelates on Metal Extraction from Contaminated Soils

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(Received on 29th August 2012, accepted in revised form 19th August 2013)

Summary: Soil contamination is the result of uncontrolled waste dumping and poor practices by human. Of all the pollutants heavy metals are of particular concern due to their atmospheric deposition, leaching capacity and non-biodegradability. Heavy metal containing effluent is discharged into the agricultural fields and water bodies. This results in the accumulation of heavy metals in soil and the crops grown on the soil. Studies have revealed detrimental impacts on soil fertility and the poor health of animals and humans. Phytoextraction is a widely researched for remediation of heavy metal contaminated soil. To enhance the effect of phytoextraction heavy metals have to be available to the plants in soluble form. In this study the potential of different chelating agents was assessed in solubilizing the heavy metals making easy for plants to uptake them. For this purpose efficient chemical and biological chelating agent had to be identified. Along with that an optimum dose and application time for chemical chelating agent was determined. Ethylenediamine tetraacetic acid (EDTA), Diethylene triamine pentaacetic acid (DTPA), Nitriloacetic acid (NTA) were applied to the soil, containing Pb, Cr, Cu and Cd, at different concentrations and application time. *Aspergillus niger* and *Aspergillus flavus* were incubated in soil for different time periods. In correspondence with findings of the study, Pb and Cr were best solubilized by 5mM EDTA. For Cd and Cu 5mM DTPA carried out efficient chelation. NTA showed relatively inadequate solubilisation, although for Cr it performed equal to EDTA. *A. niger* and *A. flavus* instead of solubilizing adsorbed the metals in their biomass. Adsorption was mainly carried out by *A. niger*.

Keywords: Chemical and biological chelates, EDTA, DTPA, NTA, phytoextraction.

Introduction

Contaminated soil

Soil contamination is the result of uncontrolled waste dumping and poor practices by human. Of all the pollutants heavy metals are of particular concern due to their atmospheric deposition, leaching capacity and non-biodegradability. Industries like iron foundries, fertilizer and textile mills are discharging their heavy metal containing effluents into the agricultural fields and water bodies and this water is being used for irrigation, polluting the soil and the food chain [1].

According to McGrath [2] the residence time of metals in soil is more than 1000 years. This leads to high accumulations, which in result would have detrimental impacts on soil fertility, proper functioning of ecosystems and would account for the poor health of animals and humans. There is now an urgent need to adopt green ways, which will guarantee economic feasibility and bringing down the contamination levels within permissible limits in a reasonable time frame. For instance using plants to extract toxins from soil is an environmental friendly solution for heavy metal contaminated soil [3].

Metal Extraction from Soil

The criteria for choosing a suitable remediation technique revolves around three empirical factors: the characteristics of contaminated location, the concentration of pollutant and the end use of contaminated medium. Efforts to treat water and soils contaminated with heavy metals are now being focused on natures self sufficiency to clean the environment for humans. Conventional techniques are now becoming inefficacious in terms of potency and cost. There are many remediation solutions like immobilization of pollutants, hydrogeological isolation of the polluted sites, electro kinetic extraction but these solutions are temporary, only restricting the scattering of pollutants rather than removing them [4].

In the recent decades one will find huge amount of evidence of the success rate of phytoextraction. Utilizing plants to decontaminate the environment is basically called bioremediation [5]. More specifically the use of plants to extract metals from water or soil is known as Phytoextraction [6].

Certain factors have to be considered while selecting the plant specie for Phytoextraction, these factors are type and amount of metal contamination, soil and climatic conditions, disposal or processing methods available for metal rich biomass, plant should be fast growing and highly tolerant to metals, easy harvest of biomass by regular agricultural techniques and the plant species should have well developed deep branched root system [7]. Due to these reasons plants like Maize (*Zea mays*), Pea (*Pisum sativa*), Sunflower (*Helianthus annuus*), Poplar (*Populussp*), Indian Mustard (*Brassica juncea*), Barley (*Hord- eumvul*gare), oat (*Avena sativ*a) and Tobacco (*Nicotiana tabacum*) have been the center of Phytoextraction [8].

Role of Chemical Chelates on Metal Extraction

Phytoavailability of the metals is a very imperative factor to achieve productive phytoextraction. The availability of metals limits the efficiency of Phytoextraction [9]. Use of some chelating agents like EDTA has shown to increase the uptake of metals by desorbing them from the solid phase of the soil due to the formation of water-soluble complexes [10]. Basically metal is partitioned into two phases: reversible and irreversible. The chelates will attempt to extract out metals first from the reversible phase and later on the irreversible one [11]. This is the criteria based on which the capability of the chelating agent is tested, that how well can they free the metals from the bound phase. The more they unlock from the irreversible phase the more efficient they are. More recently EDDS (Ethylene Diamine Disuccinate) and NTA (Nitrilo Triacetic Acid) are being employed due to their biodegradability as opposed to EDTA [12].

Chelating effect of EDTA and NTA on Cr and Pb of a contaminated soil near a stranded industrial zone was explored. Recovery of Cr was profoundly enhanced as the concentration of chelant increased. The effect of chelation was analyzed with changing pH as well so the extraction of Cr and Pb was being partly determined by pH and chelant metal chemistry as well. Approximately 100% Pb was removed at pH 4.3, 54% Cr and 96% Pb at pH 12 at 0.1M EDTA. NTA showed comparatively weaker solubilisation; 33 to 48% Cr at pH 8.9 to 11 and 38% Pb at 4.5. It is evident from this research that solubilisation of Pb is favored at acidic pH while for Cr is alkaline pH despite the fact that more leaching is done at lower pH [13].

One major downside associated with the use of chelator is the leaching of metal complexes into the groundwater. EDTA biodegrades so slowly that it is considered persistent and its addition to the soil would result in significant leaching of metals into the groundwater resources, although on the contrary this non-biodegradability also means EDTA would perform its action for a longer time [14]. EDTA heavy metal complexes have shown to be dangerous for the plants and soil microbes. This area has received a lot attention and work is in progress in search of an ecofriendly approach in using chelating agents like EDDS, which is biodegradable [15].

Role of Fungi in Metal Extraction

Besides chemically assisting phytoextraction, it can further be amplified by exploiting the synergistic relation between plants and fungus. Different fungal species protect the plants from metal toxicity and play a role in their growth and development. Zinc tolerant strains of *Suillus bovinus* (jersey cow mushroom) developed resistance in *Pinus sylvestris* (pine). Pine grown with *Pisolithus tinctorius* (Horse dung fungus) in various contaminated places exhibited tolerance to the dominant contaminant [16].

Fungi will either enhance metal uptake and phytoextraction or it will immobilize the metals and will lead to phytostabalization. These mechanisms will depend on the plant-fungi-heavy metal combination and also by local soil conditions [17]. A recent discovery provides evidence that addition of *Arbuscular Mycorrhiza fungi* (AMF) has enhanced the uptake of arsenic by *Pteris vittata* (Chinese brake). Non colonized plants accumulated 60.4mg/ kg arsenic, while colonized plants accumulated 88.1mg/ kg [18]. Another research exhibited the enhanced phytoextraction due to symbiotic coalition between AMF and the plants. This mycorrhizal fungus alters the chemical constitution of root exudates and pH of soil [19]. *Solanum nigram* was shown to increase zinc accumulation after inoculation with AMF [20].

Using soil fungus has a bright side to it, it will be tolerant to the high concentrations of toxic metals and the fungi would leach those metals at extreme pH [21]. *Aspergillus niger* has been widely utilized for bioleaching purposes. It secrets citric acid [22], oxalic acid [23] and gluconic acid [24] which acts as a chelating agent and makes the heavy metals available to plants. Bioleaching of heavy metals by the metabolites of *A. niger* was investigated. The results of the investigations showed utmost removals of 56%, 100%, 30% and 19% were for copper, cadmium, lead and zinc respectively Moreover, using biological chelates gave rise to higher heavy metal removal and low cost compared to the chemical chelates [25].

The aim of this 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 significance of this research is that it will provide an economical and green approach to remediate the soil that will be highly applicable in Pakistan. Because irrigating the fields with heavy metal contaminated water is a common practice and dearth of financial resources have always put limitations on using conventional remediation technologies.

Results and Discussion

From the data obtained it can be analyzed which chelating agent is most suitable for solubilizing metals at a particular concentration and application time and also for which heavy metal it showed maximum chelation. Moreover it can further be assessed which fungus carried out desirable activity and what time period showed highest activity. In addition to that it can be scrutinized that for which heavy metal did the fungus displayed optimum level of functioning.

Analysis of Physical Parameters of Soil

Texture: Individual sand, silt and clay content of the soil were calculated and obtained as follows: sand 14%, silt 65.5% and clay 24.5% as depicted in Fig 1. This is indicating that the soil has medium clay content as opposed to silt content.

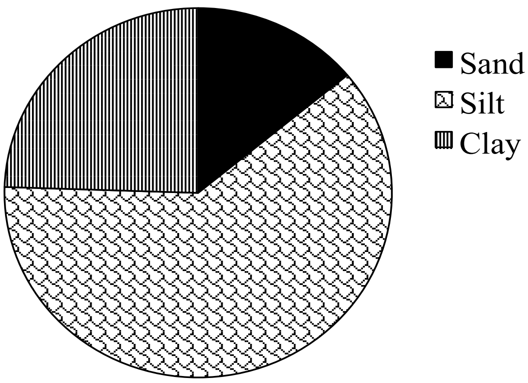

Fig. 1: Texture of soil sample of peri urban area of Multan.

The values for sand, silt and clay were applied in the soil texture triangle to determine the soil texture, which came out to be silt loam (Fig. 2).

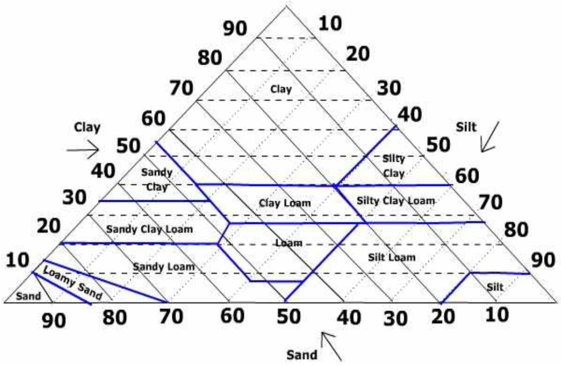

Fig. 2: Soil texture triangle.

pH, EC , carbonates and organic mater: Table 1 shows the pH of soil is 7.51 indicating that it is slightly alkaline. EC value of soil (0.147dS/m) shows that the salinity level is not very high; this is also supported by the fact that soil has high silt content and silty soils have intermediate EC. Since the carbonates are 8.92% so it can be said that the soil is calcareous. On the other hand organic matter being 0.82% would mean that the soil has low organic matter.

Table–1: Analysis of pH, EC, carbonates and organic matters of the collected soil sample of the peri-urban area of Multan.

pH	EC/dSm^{-1}	Carbonates	Organic matter
7.51	0.147	8.92%	0.82%

Analysis of Soluble Heavy Metals in Collected Soil Sample

Fig. 3 is showing the concentration of heavy metals (Cu, Cr, Cd and Pb) present in the soluble phase in the soil of control sample, which did not receive any treatment from chelates and fungi. It can be seen that Pb is present in the highest concentration while Cu is in the lowest concentration. There is not much difference in the soluble amounts of Cr and Cd.

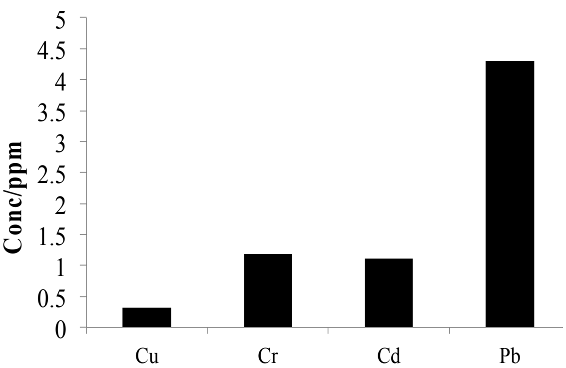

Fig. 3: Soluble metal contents in collected soil sample of peri urban area of Multan.

Analysis of Soluble Metal Contents in Chelate Treated Soil Sample

EDTA: By looking at Fig. 4a it is evident that with increasing reaction time the solubilisation of metals is slightly increasing. On the contrary Pb has shown a different pattern, EDTA has carried out maximum solubilisation in the first 24h followed by a decrease in the activity of EDTA. However compared to Cu, Cr and Cd, Pb was most solubilized. Fig 4b shows almost the same trend as shown in 4a but the increasing contact time had little influence. Moreover the solubilisation rate for all 4 metals has increased at 2.5mM EDTA.

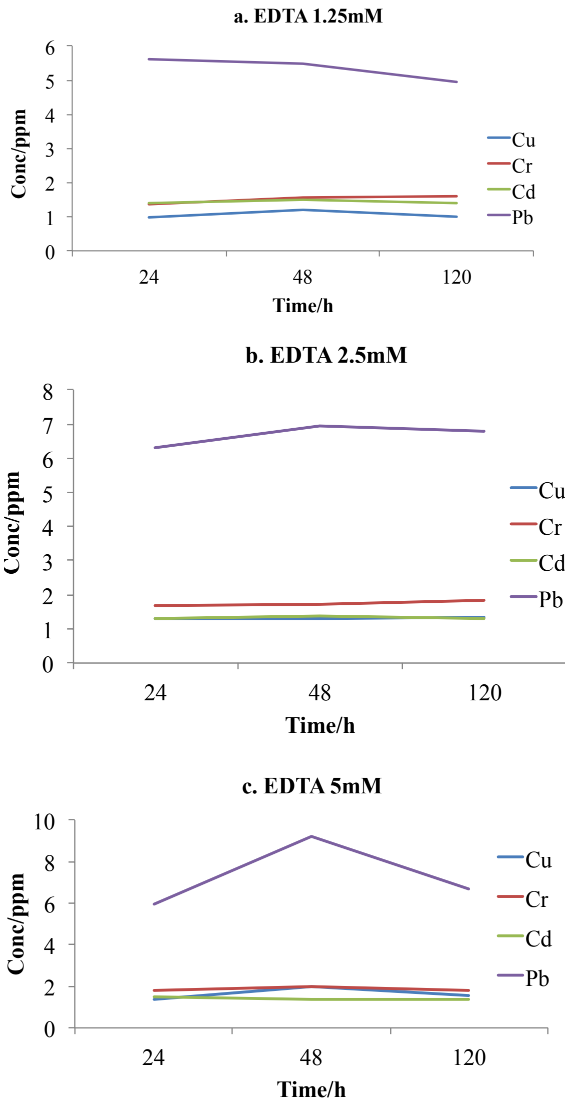

Fig. 4: (A, B, C) Solubilized metals in soil sample of peri urban area of Multan after treatment with different concentrations of EDTA against time.

At 5mM EDTA the solubilisation has moderately risen for Cu, Cr and Cd but it has stayed uniform with increasing application time. For Pb there is a hike at 48h followed by a sharp decrease.

DTPA: Similar format for Pb is observed with 1.25mM DTPA (Fig 5a), the solubilisation is constant till 48h and then there is a drop in the graph. For Cd and Cr solubilisation was carried out to a little extent; however DTPA managed to cause significant chelation of Cu relative to EDTA chelation.

Fig 5b and 5c exhibit high chelation of Pb and Cu, in addition to this, the chelation is also ascending with time. Whereas Cd and Cr are impartial towards higher concentration of DTPA and to application time as well.

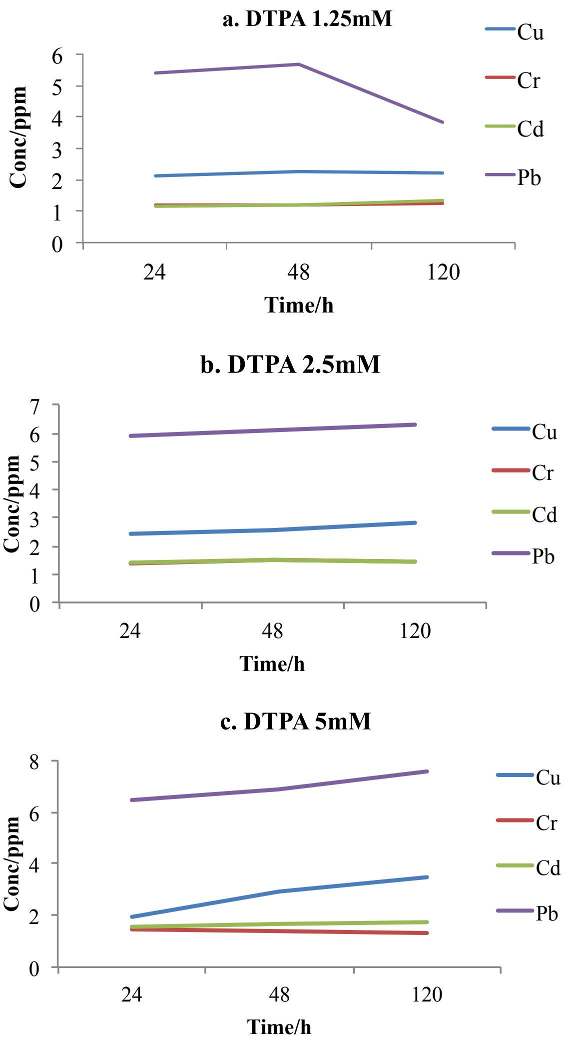

Fig. 5: (A, B, C) Solubilized metals in soil sample of peri urban area of Multan after treatment with different concentrations of DTPA against time.

<u>*NTA:*</u> NTA, relative to EDTA and DTPA, did not display very effective chelation, especially for Cu and Cr (Fig. 6a, b, c). One thing detected common in the action of NTA is that maximum chelation was done at 48h for all metals at all concentrations of NTA. However 1.25mM NTA gave higher solubilisation for Pb at 120h in fig 6a.

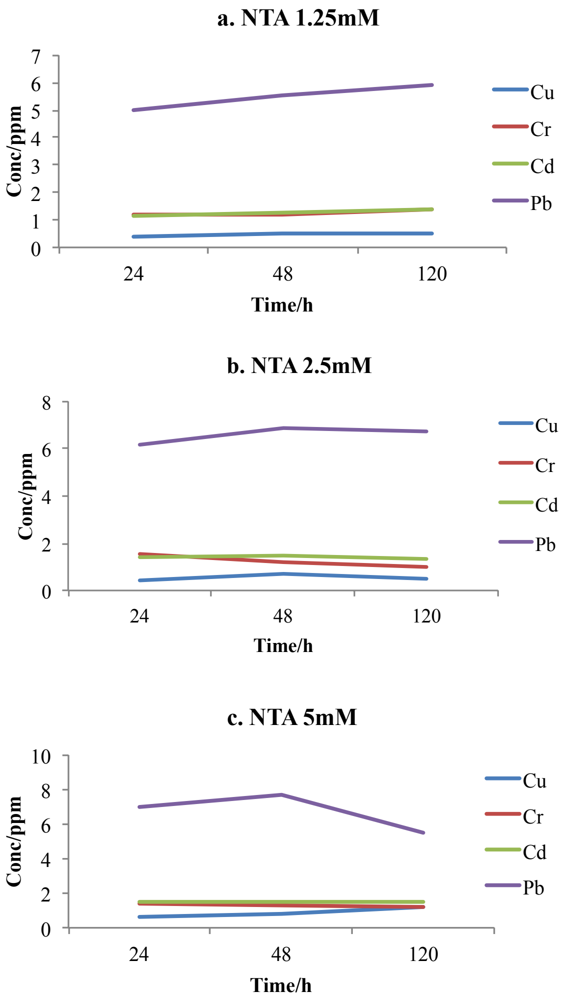

Fig. 6: (A, B, C) Solubilized metals in soil sample of peri urban area of Multan after treatment with different concentrations of NTA against time.

Fig 7 vividly depicts the maximum performance of all three chelates. It can be therefore be claimed that EDTA carried out considerable solubilisation for Pb and Cr at 5mM at 48h and DTPA gave fruitful chelation for Cu at 5mM at 120h. Incase of Cd, DTPA at 5mM at 120h takes the lead, however the functioning of NTA and EDTA cannot be overlooked.

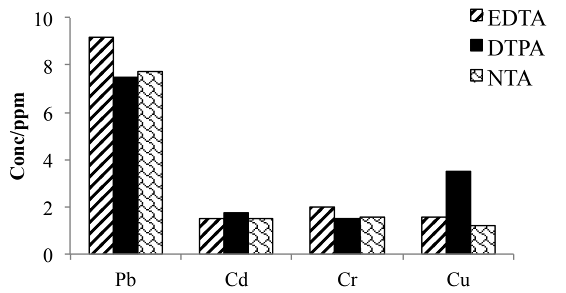

Fig. 7: Maximum Solubilisation of heavy metals in soil sample of peri urban area of Multan by EDTA, DTPA and NTA.

Examinations have been carried out to review the strength of EDTA in solubilizing Pb from the soil at lead battery recycling center [26]. 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, furthermore acidic pH favored more Pb recovery because lower pH favors leaching of metals. It was observed that as the pH was increasing Pb solubilisation was diminishing [27].

A research was performed which involved the extraction of Pb by EDTA and NTA from a soil containing 70% silt and clay. EDTA removed 68.7% of Pb and taking less time, whereas NTA removed 19.1% Pb and it took longer time 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 [28]. 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. To achieve the desired level of chelation clay content can be manipulated with.

Analysis of Soluble Metal Contents in Fungal Treated Soil Sample

Fig 8 and 9 show the solubilized heavy metals in the samples treated with fungi *A. niger and A. flavus* respectively. It is clear from the graphs that contact time between fungus and soil had little impact on the chelation of Cu, Cr and Cd. However for Pb both *A. niger* and *A. flavus* displayed different activity against time. Maximum chelation was observed at 24h for Pb by *A. niger* and *A. flavus*, but relative to the chemical chelates, fungi were not as efficient.

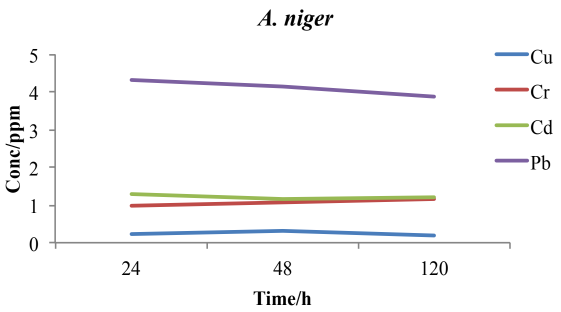

Fig. 8: Solubilized metals in soil sample of peri urban area of Multan after treatment with *A. niger* against time.

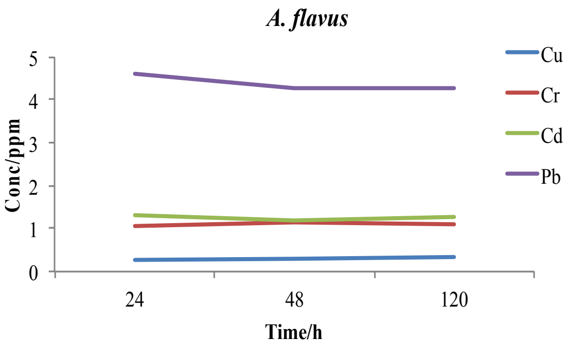

Fig. 9: Solubilized metals in soil sample of peri urban area of Multan after treatment with *A. flavus* for against time.

In correspondence with Fig 10, *A. flavus* gave substantial solubilisation for Pb, at 24h, parallel to *A. niger*, but for Cd both species performed at almost an equivalent level at 24h of treatment. Interesting information provided form fig 10 and 11 is neither of the fungal strains caused any solubilisation for Cr and adsorption of Cd. Besides solubilisation, the fungi also adsorbed the metals in their biomass (Fig. 11). *A. niger* gave a superior performance than *A. flavus* regarding the adsorption rate of Pb at 120h, Cr at 24h and Cu at 120h. It will be fair to say that fungi used in this study would be better employed for adsorption rather than for solubilisation, but either way it is remediating the soil.

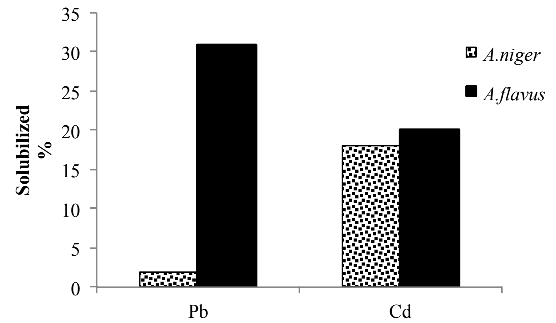

Fig. 10: Maximum Solubilisation of heavy metals in soil sample of peri urban area of Multan by *A. niger* and *A. flavus*.

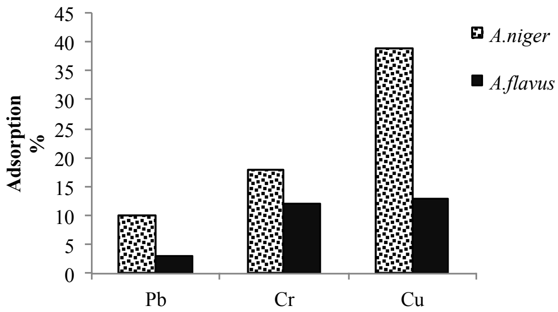

Fig. 11: Maximum adsorption of heavy metals in soil sample of peri urban area of Multan by *A. niger* and *A. flavus*

Investigations undertaken with reference to using *A. niger*, for solubilisation of metals, have focused on *A. nigers* ability to secrete citric acid with some other organic acids. These acids are then applied in soil to enhance soluble metal content [29]. On the other hand numerous studies prevail regarding the use of AM fungi with plants grown in heavy metal contaminated soil. Such a study was conducted to observe the activity of EDTA and AM fungi in helping corn and sunflower plants in up taking heavy metals. Interestingly AM fungi resulted in higher phytoextraction of Zn and Cd for both plant: corn and sunflower. This is mainly due to activities taking place in the rhizosphere, which amend the rhizospheric surroundings and the metal availability [30]. According to the researchers the performance of AM fungi was so gratifying that it could replace the need of adding EDTA to soils. Moreover experimentations give the notion that use of AM fungi, over acids extracted from *A. niger*, would be more efficient at least in terms of economics if not always in terms of competence [31].

A consistency observed in the data shows that effective chelation was observed at 48 hours, in most cases, followed by a decrease in solubilisation.

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 Ni 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 [14]. This would serve as a valid explanation as to why at 48 hours of treatment, rather than at 120 hours, most active chelation took place. Because metals were being solubilized from the storage of accessible metals and it was supported for a limited time period, after which the metals could no longer be desorbed from the soil matrix. Same trend was noticed with the solubilisation by *A. flavus* that was greatest at 24 hours of treatment. But the adsorption was highest at 120 hours.

Experimental

Study Area and Sampling

This research was conducted in Environmental Mycology and Eco-toxicology Laboratory, Department of Environmental Sciences, Fatima Jinnah Women University, Rawalpindi. Soil sample of this study was collected from peri-urban agricultural areas of Multan district. This soil is being irrigated by heavy metal contaminated water released from industries like textiles, leather, fertilizers and pesticides. This industrial setup has been operating in this area for more than 30 years. Field survey revealed that there is lack of any proper water treatment system and all the toxic wastewater is directed to agricultural fields and thus polluting it.

Analysis of Physical Parameters of Soil

pH meter and EC meter were used to measure these two parameters. To measure the texture, organic matter and carbonates of soil procedure in the provided literature was followed [32].

Treatment of Soil with Chelates for Metal Extraction

Soil sample was sieved to remove any large particles and make it uniform in texture. The soil was autoclaved 121^0C for 20 min to sterilize it or remove preexisting microbes that could interfere with the experiment [25]. Later Soil (10g) was weighed and transferred in sample bottles. Solutions of 2.5ml chelating agents (EDTA, NTA, DPTA) were added in soil with different concentrations (1.25, 2.5, 5.0mM) to evaluate their efficiency.

Treatment of Soil with Fungi for Metal Extraction

Aspergillus niger and *Aspergillus flavus* were grown in potato dextrose agar. The fungal disks of 1mm were taken from petri plates using cork borer and mixed in the soil samples. After the soil samples were kept in incubator at 30^0C for five days [25].

Analysis of Data

Periodic sampling of the soil with chelates and fungi was done after 24, 48 and 120 hours, making the overall period of 5 days. Soil sample (chelates and fungi) of 5g was weighed and mixed with 50 ml of distilled water on a shaker for 2 hours. Mixture was filtered and the soluble content of metals Cu, Cr, Cd and Pb were analyzed on AAS (Perkin Elmer Atomic Absorption Spectrometer Analyst 700 – 800).

Conclusion

The data acquired from the present experimentation provide some definite conclusions:

- From the chemical chelates EDTA seem to propose the best potential to solubilize Pb and Cr
- For Cu and Cd the effectiveness of DTPA is the highest
- Fungal species were weak in competition with the chemical chelates, but provided an alternative for remediation in form of adsorbing the heavy metals in their biomass. *A. niger* was more efficient at adsorbing Pb and Cu 120 hours of treatment and Cr 24 hours of treatment therefore *A. niger* should be employed for this purpose.
- On the other hand *A. flavus* did a better job in solubilizing Pb at 24 hours of treatment, in case of Cd, although with a narrow margin, *A. flavus* was again ahead of *A. niger* after 24 hours of treatment.
- As the results suggest fungi used in this investigation, particularly *A. niger*, would be better off at adsorbing the metals rather than to solubilize them in order to achieve remediation.

On the basis of the conclusion of present study it is recommended:

- Since pH has an imperative role towards the behavior of metals in soil, therefore experimentation should be conducted with the objective to explore the effect of pH.
- Combined effect of chemical chelates and fungi should be examined to see whether they would support each other or negate each other.

- Use two or more efficient chelates (EDTA & DTPA) in combination to analyze their integrated result.
- Use intermediate concentration of chemical chelates as the higher doses can be a threat to soil dignity and to groundwater contamination.

Acknowledgements

The authors are thankful to the Soil Science Lab of National Agricultural Research Center (NARC), Islamabad and Fatima Jinnah Women Univeristy, Rawalpindi.

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