# Research Journal of Environmental Sciences

ISSN 1819-3412

Academic
Journals Inc.

www.academicjournals.com

OPEN ACCESS Research Journal of Environmental Sciences

ISSN 1819-3412
DOI: 10.3923/rjes.2018.166.176

# Research Article
# Rhizosphere Bioremediation of Heavy Metals (Copper and Lead) by *Cenchrus ciliaris*

Pankaj Kumar and Madhusudan Hiraman Fulekar

School of Environment and Sustainable Development, Central University of Gujarat, Gandhinagar, 382030 Gujarat, India

## Abstract

**Background and Objectives:** Rhizosphere bioremediation is gaining great attention as an alternative technique for remediation of heavy metal contaminated soils. A green house study was carried out to evaluate the accumulation of heavy metals (Cu and Pb) and efficiency of grass species (*Cenchrus ciliaris*) for the remediation of heavy metals contaminated soil. **Materials and Methods:** The pot culture experiment was conducted with different concentrations of Cu (50,100,150 ppm) and Pb (20, 60,100 ppm) for 45 days. The plants were independently reaped, dried and weighed for biomass of the roots and shoots. The uptake of applied heavy metals (Pb and Cu) studied in the roots and leaf independently, to study the bioaccumulation of metals in different parts of *C. ciliaris*. The translocation variable was calculated to assess the proficiency of *C. ciliaris* for bioaccumulation of metals in roots and leaf. **Results:** The results demonstrated that *C. ciliaris* tend to accumulate more Cu and Pb metals in roots in comparison to the leaf. *C. ciliaris* accumulated Pb up to 97.31 ppm, whereas Cu accumulation was up to 188.3 ppm within 45 days. The present research work revealed *C. ciliaris* as a good accumulator of Pb and Cu productively as it effectively banished 89% of Pb and 92% of Cu individually from the contaminated soil. The bioaccumulation factor and translocation factor of *C. ciliaris* for Pb and Cu were figured out. **Conclusion:** This study concluded that rhizosphere remediation utilizing plant species *C. ciliaris* is compelling and effective green innovation for remediation of heavy metals from soil.

Key words: Heavy metals, rhizosphere bioremediation, bioaccumulation, green innovation, *Cenchrus ciliaris*

Citation: Pankaj Kumar and Madhusudan Hiraman Fulekar, 2018. Rhizosphere bioremediation of heavy metals (copper and lead) by *Cenchrus ciliaris*. Res. J. Environ. Sci., 12: 166-176.

Corresponding Author: Pankaj Kumar, School of Environment and Sustainable Development, Central University of Gujarat, Gandhinagar, 382030 Gujarat, India Tel: +91-8460571814



Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

## INTRODUCTION

Soil contamination has as of late been drawing in impressive open consideration since the extent of the issue in our soils calls for prompt activity[1]. Subsequently the human activities, such as mining and refining of metals, electroplating, gas fumes, energy and petroleum generation, fertilizer and pesticide application, which generates the heavy metals that have been considered as main contaminants of the common biological system due to their toxicity, non-biodegradation and accumulation ability. Assessing the concentration of heavy metals in the environment is a criterion for evaluating the impact of human activities on the environment. Contaminated soils with heavy metals can potentially lead to the uptake and accumulation of these metals in the edible plant parts causing a risk to human and animal health[2]. Farming practices such as fertilization and pesticide application often result in some heavy metal testimony in soils.

The remediation of metal compounds present a different set of problems as compared to organics because heavy metals are among the non-biodegradable pollutants due to their biological toxicity[3]. Rhizosphere bioremediation is one such encouraging choice that tackles the noteworthy capacities of microorganisms associated with roots to degrade organic contaminants and transform toxic metals. Since it is a plant based in-situ phytorestoration strategy, it ends up being spared, proficient and simple to execute under field conditions. Rhizoremediation forms that remove pollutants (phytoextraction, degradation and volatilization) additionally add to mitigation of poisonous qualities by diminishing the toxin fixation in the rhizosphere. Organic compounds can be degraded while metals normally need to be physically removed or be immobilized. The challenge is to develop innovative and cost-effective solutions to purify polluted environments, to make them safe for human habitat and consumption and to ensure the functioning of the ecosystems which support life. Bioremediation procedures are exceptionally appealing in contrast to the physico-chemical techniques for heavy metal expulsion from different sources in light of the fact that they are less costly and exceedingly effective even at substantially low metal concentrations[4]. Hence, these innovations should be connected to clean the heavy metals from the soil-water environment.

The present research intends to encourage the procedure of rhizoremediation of heavy metals. To achieve this aim, heavy metal tolerant plant specie was chosen for rhizoremediation experiment. The study focused on physico-chemical characterization, heavy metal characterization of experimental soil and the screening of potential plant species for remediation of selected heavy metals present in contaminated soil. The study also aimed to evaluate the phytoremediation potential of *Cenchrus ciliaris*.

## MATERIALS AND METHODS

**Experimental study area and duration:** The study was conducted at Central University of Gujarat, Gandhinagar, located 23.223°N 72.650°E in Gujarat, India. The experiment was performed during monsoon period from September to December.

**Collection of alluvial soil:** Soil used in the pot culture experiments was collected from a depth of about 0-15 cm (top soil) along the banks of the Sabarmati river basin, Gandhinagar, Gujarat. The soil was sieved, acid washed, distilled water washed and stored in a plastic bag at room temperature being used for analysis.

**Characterization of alluvial soil:** The collected soil was characterized for the different physico-chemical properties. The soil pH was measured using a digital pH meter in (1:2.5) soil to water ratio, electrical conductivity, soil moisture, water holding capacity, sulfate (Turbidimetric method), organic matter, organic carbon (Walkley and Black), total nitrogen (Kjeldahl method), available phosphorus (Olsen), potassium, analysis were performed as per the standard methods[5].

**Development of mycorrhizal soil and characterization:** Mycorrhizal soil was developed from soil based mycorrhizal inoculum at the laboratory scale. The procedure was followed as reported by Dubey and Fulekar[6]. A starter culture of mycorrhizal fungi (AMF) was obtained from the Division of Microbiology, Indian Agricultural Research Institute, New Delhi. Plastic pots of 15 cm length, perforated at the base with a capacity of 2 kg of soil were used to conduct the experiment. The collected soil was sieved through a 2 mm sieve in order to separate out the debris and dead plant material. The mixture of soil-sand in the ratio of 3:1 was prepared by mixing sterilized sand with the sieved soil, the mixture intended to provide homogeneity and porosity to the soil which would help in the growth and development of plant and its roots. One third of the starter inoculum was added to this mixture and it was properly mixed manually. Pots were placed in greenhouse in natural sunlight and controlled temperature of 23-25°C during the day and 22-24°C during night. The development of soil based mycorrhiza was checked by

Table 1: Characterization of alluvial soil, mycorrhizal soil and experimental soil

| Parameters | Alluvial soil Mean±SD | Mycorrhizal soil Mean±SD | Experimental soil Mean±SD |
|---|---|---|---|
| pH | 7.450±0.28 | 7.210±0.28 | 7.710±0.35 |
| EC (μs $cm^{-1}$) | 372.500±3.54 | 357.000±4.24 | 277.000±4.24 |
| Soil moisture (%) | 8.630±0.52 | 31.030±2.58 | 10.310±1.48 |
| W.H.C. (%) | 61.000±1.41 | 34.000±2.83 | 52.500±0.71 |
| Organic Carbon (%) | 0.295±0.02 | 0.325±0.04 | 0.335±0.04 |
| Total Carbon (%) | 0.384±0.03 | 0.423±0.05 | 0.436±0.05 |
| Organic Matter (%) | 0.507±0.04 | 0.559±0.06 | 0.576±0.06 |
| Sulphate (mg $kg^{-1}$) | 3.690±0.03 | 9.365±0.16 | 5.160±0.18 |
| Nitrogen (mg $kg^{-1}$) | 0.025±0.00 | 0.028±0.00 | 0.029±0.00 |
| Phosphorus (mg $kg^{-1}$) | 0.830±0.03 | 0.507±0.57 | 0.126±0.01 |
| Potassium (mg $kg^{-1}$) | 17.770±0.34 | 14.530±0.52 | 12.450±0.13 |

monitoring physico-chemical properties of the soil and microbiological changes in the soil and roots of the plants by sampling the rhizospheric soil and harvesting the plant roots at the periodic intervals of 1 week for a total period of 12 weeks.

**Preparation of experimental soil (alluvial+mycorrhizal +sand):** Experimental soil was prepared by the mixing of alluvial soil, mycorrhizal soil and sand in the ratio of 3:1:1. Further, it was characterized for physico-chemical properties like pH, electrical conductivity, soil moisture, water holding capacity, sulfate (Turbidimetric method), organic matter, organic carbon (Walkley and Black method), total nitrogen (Kjeldahl method), available phosphorus (Jackson-Murphey), potassium, analysis were performed as per the standard methods[5]. Characterization of alluvial soil, mycorrhizal soil and sand presented in Table 1.

**Collection of seeds for experiment:** The seeds of different grasses for study purpose, *Cenchrus biflorus* (Dhaman grass), *Panicum maximum* (Guinea grass), *Hetropogon* sp. (Tangle grass), *Cenchrus ciliaris* (Buffel grass) were collected from Seed technology department, Indian Grassland and Fodder Research Institute, Jhansi, Uttar Pradesh, India.

**Screening of plant species for tolerance of heavy metals (Pb and Cu):** All collected seeds were tested for their tolerance to different concentration of lead i.e., 20, 60 and 100 ppm and copper i.e., 50, 100 and 150 ppm. The salt solution of Pb [lead acetate, $Pb(C_2H_3O_2)_2$] and Cu [copper sulphate, $CuSO_4$] were prepared. About 200 gm sieved soil was filled into plastic pots. Soil was spiked with Pb and Cu as per the treatment schedule. The spiked soil was kept ventilated for 24 h. Later, the seeds were sown (10 seeds per pot) and the pots were maintained in a greenhouse and their growth monitored. The viability of seeds was checked by the standard germination test (ISTA[7]):

$$\text{Germination (\%)} = \frac{\text{Number of seeds germinated}}{\text{Total number of seeds sown}} \times 100$$

**Experimental design and treatment:** Small pots of 2 kg capacity were filled with approximately 2 kg experimental soil amended with different concentration of heavy metals. *C. ciliaris* was selected for the experiment and the seeds were sown in the pots with Pb (20 ppm $\{T_1\}$, 60 ppm $\{T_2\}$ and 100 ppm $\{T_3\}$) and Cu (50 ppm $\{T_1\}$, 100 ppm $\{T_2\}$ and 150 ppm $\{T_3\}$) and observed in 15, 30 and 45 days after sowing. The research study was being carried out upto 45 days.

**Rhizosphere bioremediation-greenhouse experiment:** *C. ciliaris* was grown in soil amended with Pb (20, 60 and 100ppm) and Cu (50, 100 and 150 ppm). The rhizosphere bioremediation of Pb and Cu was carried up to the period of 45 days. Pots were filled with soil mix [soil and sand mixture (<2 mm) 3: l (w/w) along with about 20% mycorrhizal (VAM) inoculum] spiked at various concentrations and kept in a greenhouse. Soil samples were collected at three intervals of 15, 30 and 45 days to assess the rhizospheric degradation of Pb and Cu.

**Examination of soil and plant samples during the experiment:** Soil samples were analyzed for their physico-chemical properties and heavy metals (Pb and Cu). Plant samples were also analyzed for determining the heavy metal concentration accumulated by the roots and leaves in rhizospheric ecosystem at 15, 30, 45 days, respectively using Atomic Absorption Spectroscopy. The plant materials were harvested without harming the roots and washed in distilled water to remove, sediment and mineral particles. These were then separated into roots and leaves and dried at 80°C for 48 h in a hot air oven to a constant weight and kept in sealed plastic bags for metal analysis.

Table 2: Percentage germination of seeds in various concentrations of Lead (Pb)

| | Germination (%) for lead | | |
|---|---|---|---|
| Seeds of grass | 20 ppm | 60 ppm | 100 ppm |
| *Cenchrus biflorus* | 40 | 30 | 50 |
| *Panicum maximum* | 30 | 20 | 40 |
| *Hetropogon* sp. | 50 | 30 | 60 |
| *Cenchrus ciliaris* | 80 | 70 | 70 |

Table 3: Percentage germination of seeds in various concentration of Copper (Cu)

| | Germination (%) for copper | | |
|---|---|---|---|
| Seeds of grass | 50 ppm | 100 ppm | 150 ppm |
| *Cenchrus biflorus* | 30 | 20 | 40 |
| *Panicum maximum* | 50 | 30 | 60 |
| *Hetropogon* sp. | 40 | 60 | 40 |
| *Cenchrus ciliaris* | 90 | 70 | 80 |

**Screening of Grass species for remediation of heavy metals (Pb and Cu):** Several concentrations were prepared for both heavy metals i.e., for Pb (20, 60, 100 ppm) and Cu (50, 100, 150 ppm). After a week of sowing, germination of seeds was observed, the percentage of seed germination was calculated by dividing the number of germinated seeds by the total number of seeds. Among the 4 species of grasses, seeds of *C. ciliaris* (Buffel grass) provided 70 and 80% germination in higher concentration of both heavy metals (Table 2 and 3). On this basis of the germination results, *C. ciliaris* was selected for further rhizoremediation of heavy metals.

**Rhizoremediation of heavy metals:** Rhizoremediation of the soil containing metals has been completed under controlled ecological conditions. The rhizosphere bioremediation of Pb and Cu was carried up to a period of 45 days. The pots were observed every 15 days after sowing the seeds. The physico-chemical characterization was also done at every 15th, 30th and 45th day.

**Specification of fungi and bacteria counts:** Enumeration of bacteria started within 5-6 h of collection using a serial dilution technique. The enumeration of bacteria and fungi were done by the serial dilution method or viable plate count method[8]. One gram of soil was mixed with 10 mL of sterile distilled water. After dilution, the sample was added to petri dishes containing a growth medium consisting agar mixed with selected nutrients. An aliquot of 0.1 mL of dilutions for each soil sample was spread plated onto agar plates from the appropriate dilution tubes and incubated at room temperature. The bacterial colonies were counted after every 24 h. The results were averaged for each soil sample. The fungal colonies were counted after 48-72 h.

CFU $mL^{-1}$ can be calculated using the formula:

$$\text{CFU mL}^{-1} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Volume of culture plate}}$$

**Bioaccumulation factor:** Heavy metal concentrations in soils and plants were calculated on the basis of dry weight. The bioaccumulation factor (BF), an index of the ability of the plant to accumulate a particular metal with respect to its concentration in the soil substrate[2], was calculated as follows:

$$BF = \frac{C_{plant}}{C_{soil}}$$

Where, $C_{plant}$ and $C_{soil}$ represent the heavy metal concentrations in the plant and soil, respectively.

**Translocation factor:** Translocation factor (TF) was calculated to define the relative translocation of metals from soil to root and leaf of the plant. TF is the ratio of metal concentration in the shoots to the roots ([Metal] Shoot/[Metal] Root)[9,10].

**Statistical analysis:** The data collected through the experiments has been classified and mean values were tabulated for analysis. The differences among the treatments were observed and represented in the form of charts, tables and bar-graphs with the help of Microsoft office, 2013.

## RESULTS AND DISCUSSION

**Physico-chemical properties of alluvial, mycorrhizal and experimental soil:** Alluvial soil was collected from the Sabarmati river basin, Mycorrhizal soil was developed using mycorrhizal inoculum and sorghum species and experimental soil prepared by mixing of alluvial soil, mycorrhizal soil and sand in the ratio of 3:1:1 exhibited pH values above neutral

Table 4: Physico chemical Characterization of Lead (Pb) amended soil after 15, 30 and 45 days of treatment

| | $T_1$ (20 ppm) | | | $T_2$ (60 ppm) | | | $T_3$ (100 ppm) | | |
|---|---|---|---|---|---|---|---|---|---|
| Parameters | 15 days | 30 days | 45 days | 15 days | 30 days | 45 days | 15 days | 30 days | 45 days |
| pH | 8.12 | 8.26 | 8.32 | 8.03 | 8.10 | 8.39 | 8.31 | 8.34 | 8.36 |
| EC (µs $cm^{-1}$) | 880 | 940 | 1110 | 1160 | 1420 | 1380 | 19900 | 9500 | 1050 |
| Soil moisture (%) | 14.56 | 16.5 | 17.2 | 10.6 | 11.9 | 13.5 | 9.6 | 10.2 | 8.5 |
| W.H.C. (%) | 54.5 | 56.2 | 58.5 | 41.5 | 46.6 | 47.4 | 48 | 51.5 | 53.2 |
| Organic carbon (%) | 0.29 | 0.32 | 0.35 | 0.39 | 0.42 | 0.46 | 0.43 | 0.39 | 0.41 |
| Total carbon (%) | 0.377 | 0.416 | 0.455 | 0.507 | 0.546 | 0.598 | 0.558 | 0.507 | 0.533 |
| Organic matter (%) | 0.49 | 0.56 | 0.602 | 0.67 | 0.72 | 0.79 | 0.739 | 0.67 | 0.7052 |
| Total nitrogen (%) | 0.0245 | 0.028 | 0.0301 | 0.0335 | 0.036 | 0.039 | 0.036 | 0.033 | 0.035 |
| Phosphorus (mg $kg^{-1}$) | 0.96 | 1.01 | 1.079 | 0.92 | 0.87 | 0.8 | 0.8 | 0.85 | 0.809 |
| Potassium (mg $kg^{-1}$) | 10.3 | 11.03 | 11.19 | 15.32 | 11.9 | 16.36 | 18.88 | 14.56 | 9.84 |

Table 5: Physico chemical Characterization of Copper (Cu) amended Soil after 15, 30 and 45 days of treatment

| | $T_1$ (50 ppm) | | | $T_2$ (100 ppm) | | | $T_3$ (150 ppm) | | |
|---|---|---|---|---|---|---|---|---|---|
| Parameters | 15 days | 30 days | 45 days | 15 days | 30 days | 45 days | 15 days | 30 days | 45 days |
| pH | 8.01 | 8.20 | 8.34 | 8.360 | 8.75 | 8.64 | 8.21 | 8.26 | 8.30 |
| EC (µs $cm^{-1}$) | 450 | 750 | 830 | 650 | 840 | 960 | 1090 | 860 | 540 |
| Soil moisture (%) | 9.60 | 14.50 | 16.60 | 1.230 | 16.50 | 14.20 | 9.25 | 11.20 | 10.50 |
| W.H.C. (%) | 62.50 | 59.50 | 56.20 | 52.100 | 56.30 | 54.50 | 47.50 | 48.30 | 45.50 |
| Organic carbon (%) | 0.47 | 0.37 | 0.24 | 0.580 | 0.46 | 0.41 | 0.41 | 0.34 | 0.27 |
| Total carbon (%) | 0.65 | 0.56 | 0.312 | 0.754 | 0.598 | 0.533 | 0.533 | 0.456 | 0.351 |
| Organic matter (%) | 0.96 | 0.54 | 0.41 | 0.997 | 0.791 | 0.705 | 0.705 | 0.590 | 0.464 |
| Total nitrogen (%) | 0.048 | 0.027 | 0.0205 | 0.049 | 0.0395 | 0.0352 | 0.035 | 0.029 | 0.023 |
| Phosphorus (mg $kg^{-1}$) | 1.80 | 1.25 | 0.944 | 1.650 | 1.10 | 0.95 | 2.42 | 2.350 | 2.42 |
| Potassium (mg $kg^{-1}$) | 17.50 | 13.20 | 9.01 | 14.650 | 11.56 | 10.89 | 15.91 | 12.890 | 10.11 |

and electrical conductivity of these soils varied from 372.5-277 µs $cm^{-1}$. Organic carbon and Total nitrogen content varied slightly among the alluvial soil and prepared soil for experiment. Available phosphorus and potassium showed a gradual decreasing trend among the soil (Table 1). The alluvial soil also characterized to evaluate the background concentration of lead (9.92 ppm), copper (69.67 ppm) and zinc (50.98 ppm) to facilitate the experimental soil preparation.

**Germination percentage of seeds in different concentration of lead and copper:** Seeds were exposed to grow with different concentrations of heavy metals (Pb and Cu) as mentioned in the methodology and observed that *Cenchrus ciliaris* (Buffel grass) gave 70% and 80% germination in higher concentration of both heavy metals (Table 2 and 3).

**Physico-chemical characterization of lead amended soil:** The Table 4 illustrated variation in soil characteristics during the experiment which exhibited variationin pH and electrical conductivity among the treatments. The changes were also observed in organic carbon, total nitrogen, available phosphorus and potassium among the treatments. The total nitrogen content and potassium showed a similar trend of increase in $T_1$ and $T_2$ whereas decresed in $T_3$. Available phosphorus exhibited a variation from 0.96-1.079 mg $kg^{-1}$ in $T_1$, while in $T_2$ it decreased from 0.92-0.8 mg $kg^{-1}$. In $T_3$ it, increased from 0.8-0.85 mg $kg^{-1}$ and then it decreased to 0.809 mg $kg^{-1}$.

**Physico-chemical characterization of copper amended soil:** The role of copper in plants as micronutrients is known and here assessed it in terms of copper released from external sources in higher amounts. In present observation, copper (Cu) treatment on the soil exhibited increased pH with time and it varied from 8.01-8.34 in $T_1$ to 8.21-8.3 in $T_3$. EC was similarly observed at regular intervals and it was observed that in $T_1$ and it increased with time whereas in $T_3$, it was significantly decreased. Organic carbon and macronutrient content (N,P,K) in the soil decreased during the study period (Table 5).

Macronutrients like nitrogen, phosphorus and potassium are essential for plant growth as they serve as a major building blocks as well as co-factors for various enzymes in metabolic pathways in plants and microorganisms. Increase in phosphorus content indicates the high microbial activities during the rhizoremediation process which causes more mineralization. Plants deliver root exudates of carbon, energy, vitamins, enzymes and from time to time oxygen to the microbial population in the rhizosphere. Those exudates offer

Table 6: Microbial characterization in Pb amended soil

| Study periods | 20 ppm | 60 ppm | 100 ppm |
|---|---|---|---|
| **Bacteria (CFU mL$^{-1}$)** | | | |
| 15 days | $1.76\times10^{7}$ | $1.71\times10^{7}$ | $1.62\times10^{7}$ |
| 30 days | $1.86\times10^{7}$ | $1.81\times10^{7}$ | $1.79\times10^{7}$ |
| 45 days | $1.70\times10^{7}$ | $1.68\times10^{7}$ | $1.61\times10^{7}$ |
| **Fungi (CFU mL$^{-1}$)** | | | |
| 15 days | $1.62\times10^{5}$ | $1.58\times10^{5}$ | $1.48\times10^{5}$ |
| 30 days | $1.71\times10^{5}$ | $1.67\times10^{5}$ | $1.63\times10^{5}$ |
| 45 days | $1.53\times10^{5}$ | $1.46\times10^{5}$ | $1.41\times10^{5}$ |

Table 7: Microbial characterization in Cu amended soil

| Study periods | 50 ppm | 100 ppm | 150 ppm |
|---|---|---|---|
| **Bacteria (CFU mL$^{-1}$)** | | | |
| 15 days | $2.21\times10^{7}$ | $2.18\times10^{7}$ | $1.97\times10^{7}$ |
| 30 days | $2.31\times10^{7}$ | $2.14\times10^{7}$ | $2.20\times10^{7}$ |
| 45 days | $2.15\times10^{7}$ | $2.01\times10^{7}$ | $2.00\times10^{7}$ |
| **Fungi (CFU mL$^{-1}$)** | | | |
| 15 days | $1.71\times10^{5}$ | $1.61\times10^{5}$ | $1.60\times10^{5}$ |
| 30 days | $1.81\times10^{5}$ | $1.79\times10^{5}$ | $1.74\times10^{5}$ |
| 45 days | $2.00\times10^{5}$ | $1.97\times10^{5}$ | $2.13\times10^{5}$ |

enough carbon to assist the a wide variety of microbes within the rhizosphere and plant triggered enrichment of the microbial population is referred to as rhizosphere impact[11]. The decrease in total organic carbon in the pots could be attributed to the usage of organic carbon via plant life and microbes. As a supplementary compartment, plant roots can engage with microbe and organic contaminants. The physical conditions that have an effect on metal bioavailability are temperature and moisture. In the current study, increase in moisture content elevated bioavailability of heavy metals in plant treatments which improved the rate of deterioration and hyper accumulation. Bioavailability of pollutant is additionally enriched by chelators that are released in the rhizosphere by plants and bacteria. Chelators such as siderophore, organic acids and phenolic compounds can release metal cations from soil, which make the metals more available for plant uptake.

**Microbial status:** Microbiological activities are important in determining metal transportability and have actual and potential application in bioremediation of metallic pollution. These encompass autotrophic and heterotrophic leaching mechanisms, reductive precipitation, sulfate diminishment and metal sulfide precipitation[12]. Table 6 and 7 showed the bacterial and fungal count during rhizosphere bioremediation of Pb and Cu.

Numerous microbial species are known to be fit for adsorbing heavy metals on their surfaces and additionally amassing inside their structures[13,14]. It has been demonstrated that microscopic organisms and parasites found in contaminated environment are more tolerant to elevated amounts of metals than those found in unpolluted territories and that tolerant microorganisms are found in higher population in polluted natural surroundings[15,16]. Since heavy metal uptake and tolerance depend on both plant and soil factors, including soil microbes, were quire information on interactions between plant roots and their symbionts such as arbuscular mycorrhizal (AM) fungi and nitrogen fixing microbes. It is the generally held view that the majority of plants growing under natural conditions has mycorrhizae[17]. Mycorrhizal colonization of roots results in an increase in root surface area for nutrient acquisition. The extra metrical fungal hyphae can extend several cm into the soil and uptake large amounts of nutrients, including heavy metals, to the host root.

Symbiosis between plants and microbes in the rhizosphere has long been studied by microbial ecologists[18]. The rhizosphere is an area encircling the plant root system, which is characterized by enhanced biomass productivity. Rhizosphere bacteria obtain nutrients excreted from rats, such as organic acids, enzymes, amino acids and complex carbohydrates[8]. Further, rhizosphere microbes play significant roles in reprocessing of plant nutrients, maintenance of soil structure, detoxi cation of noxious chemicals and control of plant pests[19,20]. On the other hand, the plant root exudates offer nutrition to rhizosphere microbes, thus increasing microbiological activity in the rhizosphere, which in turn, stimulate plant growth and reduce the metal toxicity in plants.

**Concentration of lead (Pb) and copper (Cu) accumulation in soil, roots and leaves:** The accumulation of lead (Pb) and copper (Cu) in different treatments of soil and different

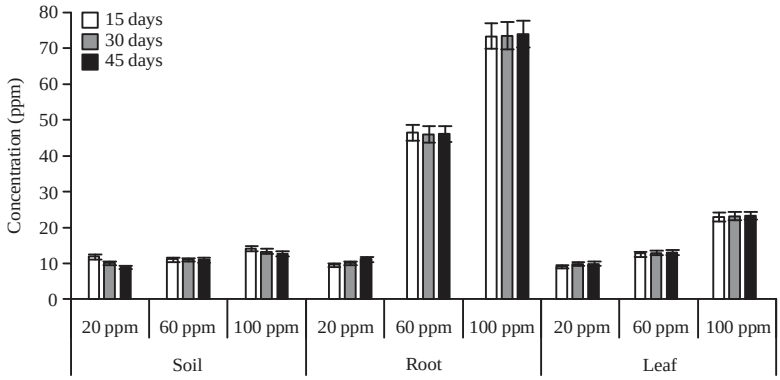


Fig. 1: Accumulation of Pb in soil, root and leaf of *Cenchrus ciliaris*

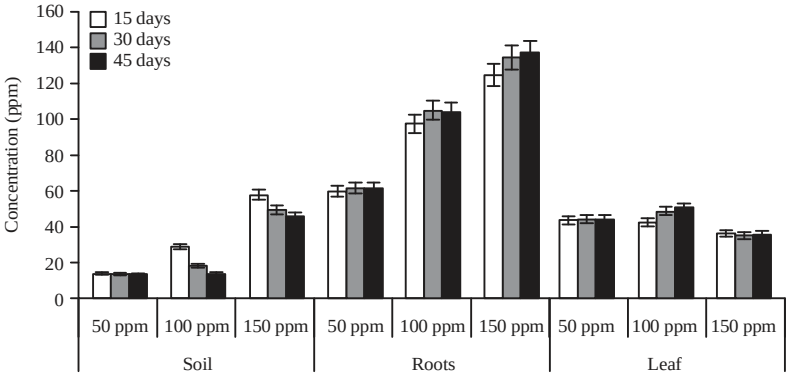


Fig. 2: Accumulation of Cu in soil, root and leaf of *Cenchrus ciliaris*

plant tissue (roots and leaves) of *C. ciliaris* were shown in Fig. 1 and 2. The concentration of Pb and Cu in the different parts of the plants harvested from the different treatments showed a high level of metal accumulation in the roots in comparison to the leaves.

The uptake of Pb and Cu by *C. ciliaris* was higher in roots than in leaves (roots >leaves). A general increasing trend in metal accumulation occurred with the increasing metal concentrations in soil with time. The maximum concentrations of Pb and Cu reached 74.01 ppm, 137.5 ppm in the roots and 23.3 ppm, 50.8 ppm in the leaf of *C. ciliaris*, respectively. Certain plants (mostly, belonging to the Brassicaceae, Euphorbiaceae, Asteraceae, Lamiaceae and Scrophulariaceae families) have been recognized which have the potential to uptake Pb[21,22]. In most soils, Pb has low geochemical mobility and low bioavailability. Furthermore, the transport of Pb to above ground is minimal due to its retention in plant roots by sorption and precipitation.

Nazir *et al.*[23] reported significant accumulation of different heavy metal in root of Cenchrus spp. growing in industrial contaminated areas. The highest fungicidal potential of methanolic root might be related with occurrence of diversity of sterols, such as cycloergost, phytol and β-tocopherol in root extract of different Cenchrus spp.[24,25]. Additionally, C. *ciliaris*, is a hyper-root-accumulator of heavy metal and could be used for phytoremediation purpose[26,27]. Sun *et al.*[28] identified *Cenchrus ciliaris* as suitable plants to be utilized for bioremediation in surface saline soil or marine sediments, for its ability to grow in soil with (1-2% NaCl).

*Cenchrus ciliaris* (C4) is picking up consideration in different fields of research, as this is most appropriate to the present natural condition. C4 grasses are more aggressive under the state of high temperature, sunlight based radiation and low moisture[29]. This grass is more proficient at gathering $CO_2$ and utilizing nitrogen from the atmosphere and recycled in the soil[30]. This grass has excellent soil binding capacity which helps to conserve soil in desert areas. However, *C. ciliaris* is most appropriate and exceedingly nutritive grass for betray ecological conditions[24].

Qian *et al.*[31] have reported that the highest Pb concentrations in both shoot (64 mg $kg^{-1}$) and roots (1882 mg $kg^{-1}$) were attained by smart weed. Parrot's feather, umbrella plant *(Cyperus alternifolius* L.), fuzzy water clover *(Marsilea drummondii*) accumulated Pb levels ranging from

30-45 mg $kg^{-1}$ in shoots and 1000-1200 mg $kg^{-1}$ in roots. They indicated that lead uptakes of other plants viz., sedge *(Cyperus pseudovegetus*), smooth cord grass, monkey flower *(Mimulus guttatus* Fisch.), mare's tail *(Hippuris vulgaris* L.) and iris-leaved rush (*Juncus xiphioides E. Mey.*) did not exceed 18 mg $kg^{-1}$ in shoots and 200 mg $kg^{-1}$ in roots.

Muramoto and Oki[32] found that water hyacinth accumulated 25800 mg Pb $kg^{-1}$ when it was treated with 8 mg Pb $L^{-1}$. Shoot of Indian mustard grown on contaminated soils (total Pb 31000 mg $kg^{-1}$) treated with 160 mmol S $kg^{-1}$ and EDTA had 7100 mg Pb $kg^{-1}$, whereas wheat accumulated 1095 mg Pb $kg^{-1}$ at the same treatment.

The availability, stability and fate of copper in this form depend on various physico-chemical factors, e.g., soil pH and chemical form. A general observation was that higher copper concentrations were found in plants in the higher copper concentration soils. The grasses had constantly higher copper concentrations than tree foliage at the same copper soil concentration, as determined from the copper analyses of washed plant material. These observations are supported by the results described by Hutchinson[33].

The grasses, *Cenchrus ciliaris* and *Enneapogon cenchroides*, both being palatable species for wild herbivores contained the highest copper concentrations. Wild[34] also measured high copper concentrations in these species in copper rich soils in Zimbabwe but also reported the presence of various accumulator species or cuprophiles, none of which could be found within the study area.

**Bioaccumulation factor for Pb and Cu:** Bioaccumulation can be defined as the plant's ability to accumulate metals from soils and can be estimated using the Bioaccumulation factor (BF). In the present research study, the Bioaccumulation factor (BF) represented the ability of *C. ciliaris* to extract heavy metals from the metal amended soil. The bioaccumulation factor from soil to leaf was indicated as BF (A) and from soil to root as BF (B) in Fig. 3.

The bioaccumulation factor for 20 ppm lead amended soil on 15th day (0.76-soil to leaf, 0.78-soil to root), on 30th day (0.98-soil to leaf, 1-soil to root), on 45th day (1.11-soil to leaf, 1.25-soil to root). For 60 ppm lead amended soil on 15th day (1.13-soil to leaf, 4.18-soil to root), on 30th day (1.18-soil to leaf, 4.20-soil to root), on 45th day (1.22-soil to leaf, 4.30-soil to root). And for 100 ppm lead amended soil BF on 15th day (1.64-soil to leaf, 5.26-soil to root), on 30th day (1.74-soil to leaf, 5.53-soil to root), on 45th day (1.85-soil to leaf, 5.87-soil to root).

Bioaccumulation factor for 50 ppm copper amended soil on 15th day (3.15-soil to leaf, 4.31-soil to root), on 30th day (3.24-soil to leaf, 4.50-soil to root), on 45th day (3.31-soil to leaf, 4.63-soil to root). For 100 ppm copper amended soil on 15th day (1.48-soil to leaf, 3.38-soil to root), on 30thday (2.68-soil to leaf, 5.80-soil to root), on 45th day (3.64-soil to leaf, 7.50-soil to root). And for 150 ppm copper amended soil BF on15th day (0.63-soil to leaf, 2.15-soil to root), on 30th day (0.71-soil to leaf, 2.72-soil to root), on 45th day (0.78-soil to leaf, 2.98-soil to root).

**Translocation factor for Pb and Cu:** Metals that are accumulated by plants are largely stored in the roots of plants and are designated as translocation factor (TF). The translocation factor from soil to root is indicated as TF (A), from soil to leaf as TF (B) and from root to leaf as TF (C) in Fig. 4. The translocation factor for 20 ppm lead amended soil on 15th day (0.78-soil to root, 0.76-soil to leaf, 0.97-root to leaf), on 30th day (1-soil to root, 0.98-soil to leaf, 0.97-root to leaf), on 45th day (1.25-soil to root, 1.11-soil to leaf, 0.89-root to leaf).

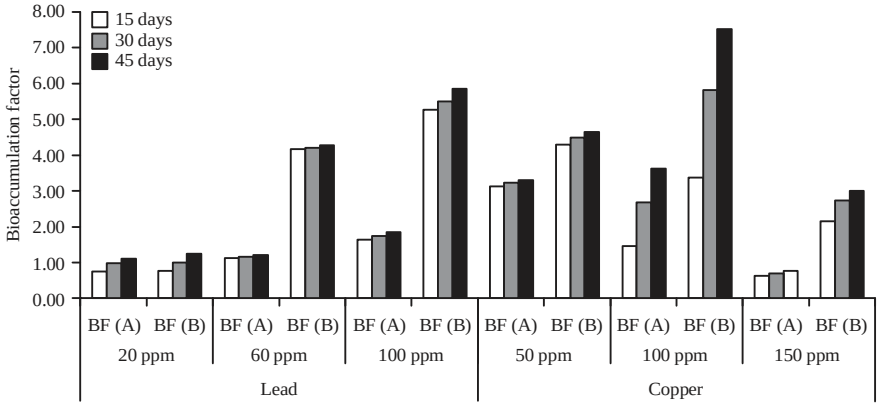


Fig. 3: Bioaccumulation factor for Pb and Cu in different parts of *Cenchrus ciliaris* *BF (A): Bioaccumulation factor from soil to leaf, BF (B): Bioaccumulation factor from soil to root

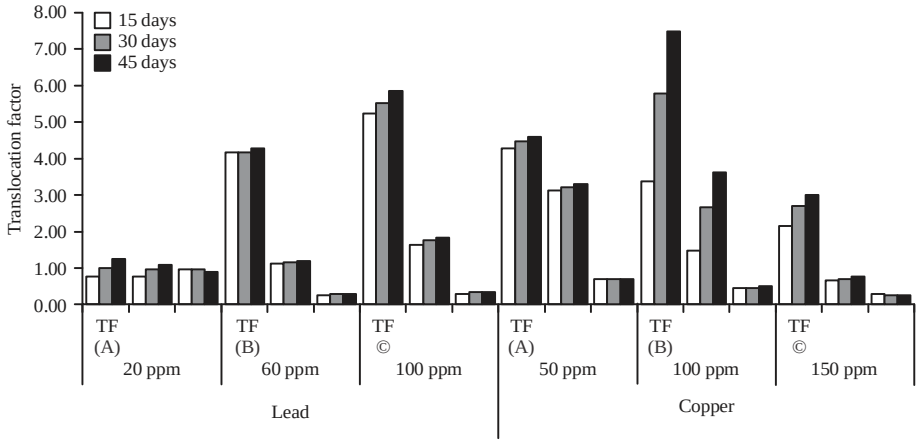


Fig. 4: Translocation factor for Pb and Cu in different parts of *Cenchrus ciliaris*, *TF (A): Translocation Factor from soil to root, TF (B): Translocation factor from soil to leaf, TF (C): Translocation factor from root to leaf

For 60 ppm lead amended soil on 15th day (4.18-soil to root, 1.13-soil to leaf, 0.27-root to leaf), on 30th day (4.20-soil to root, 1.18-soil to leaf, 0.28-root to leaf), on 45th day (4.30-soil to root, 1.22-soil to leaf, 0.28-root to leaf). And for 100 ppm lead amended soil TF on 15th day (5.26-soil to root, 1.64-soil to leaf, 0.31-root to leaf), on 30th day (5.53-soil to root, 1.74-soil to leaf, 0.32-root to leaf), on 45th day (5.87-soil to root, 1.85-soil to leaf, 0.31-root to leaf).

The translocation factor for 50 ppm copper amended soil on 15th day (4.31-soil to root, 3.15-soil to leaf, 0.73-root to leaf), on 30th day (4.50-soil to root, 3.24-soil to leaf, 0.72-root to leaf), on 45th day (4.63-soil to root, 3.31-soil to leaf, 0.71-root to leaf). For 100 ppm copper amended soil on 15th day (3.38-soil to root, 1.48-soil to leaf, 0.44-root to leaf), on 30th day (5.80-soil to root, 2.68-soil to leaf, 0.46-root to leaf), on 45th day (7.50-soil to root, 3.64-soil to leaf, 0.48-root to leaf). And for 150 ppm copper amended soil TF on15th day (2.15-soil to root, 0.63-soil to leaf, 0.29-root to leaf), on 30th day (2.72-soil to root, 0.71-soil to leaf, 0.26-root to leaf), on 45th day (2.98-soil to root, 0.78-soil to leaf, 0.26-root to leaf).

The highest bioaccumulation factor for Pb was found 1.85 (soil to leaf) and 5.87 (soil to root) in $T_3$ at 45th day in leaf of *C. ciliaris*. Bioaccumulation factor for Cu was higher 5.80 (soil to root ) in $T_2$ at 30th day. The translocation factor for Pb was highest in $T_3$ at 45th day 5.87 (soil to root). And for Cu it was found highest in $T_2$ at 45th day 7.50 (soil to root). The expansion in BF with time was in concurrence with literature[20]. The translocation property portrayed that the content of heavy metals collected in the shoot (counting stems and leaves) of a planet ought to be higher than those in its roots, i.e., TF>1. Different concentrations of metals were accumulated in leaves that might be depend upon water-soil quality. According to a previous study, pH played a major role in the bioaccumulation of metals. An increase in metal uptake with an increase in soil metal concentrations during the early developmental stages was observed which indicates the plant's tolerance to the heavy metals (Pb and Cu). But a decrease in metal uptake with the maturity of the plant was observed. This indicates that the plant's ability for the metal uptake reduces with time i.e. with maturity.

**Removal efficiency of *C. ciliaris* for the remediation of Pb and Cu:** Based on the monitoring data collected, it could be concluded that the plant species *Cenchrus ciliaris* showed significant accumulation of both the heavy metals in soil. The results revealed increasing tendency of removal with the increasing time period. The results achieved from the present study indicated that highest metals removal percentage for Pb was 89% at 100 ppm lead treatment at 45th day and for Cu highest percentage was 92% at 100 ppm copper treatment at 45th day.

*Cenchrus ciliaris* was able to eradicate 61-89% of Pb, 74-92% of Cu in 45 days treatment period. The removal efficiencies for Pb and Cu vary with varying concentration of heavy metals treatments. The removal percentage for Pb in $T_1$ 61% at 15th day, 67% at 30th day and 71% at 45th day. In $T_2$ the removal percentage, approximately 85% from 15th to 45th day. In $T_3$ the removal percentage 88% at 15th day, 88% at 30th day and 89% at 45th day respectively. The removal percentage for Cu in $T_1$ almost 89% from 15th to 45th day. In $T_2$ the removal percentage 83% at 15th day, 90% at 30th day and 92% at 45th day. In $T_3$ the removal percentage 74% at 15th day, 78% at 30th day and 79% at 45th, day respectively. The study correlates the efficiency of *Cenchrus ciliaris* in metal removal from experimental soil in mycorrhizal

association. The rhizosphere bioremediation can be implemented in contaminated soil using grass *Cenchrus ciliaris.* The study was carried out in green house conditions which need to be tried at field for more efficient outcomes and survival in the seasonal variation. The study encourages utility of different types of fodder grasses for bioremediation in future and their utility in mixed farming system at contaminated sites.

## CONCLUSION

The current research study proved that *C. ciliaris* is a virtuous accumulator of Pb and Cu successfully and survives in polluted soil. On the basis of outcomes, *C. ciliaris* can be advocated for the elimination of heavy metals (Pb and Cu) from polluted soil. The BF's of the shoots and roots and TF's being >1 shows the validity of the *C. ciliaris* for hyper accumulation of the metal Pb and Cu. This is due to the plant's capability to uptake the metal and its tolerance capacity for the heavy metal Pb and Cu. Hence, it can be a promising species for rhizoremediation of heavy metals and remediation of contaminated soils which is efficient and ecofriendly.

## ACKNOWLEDGMENT

The author is grateful to the School of Environment and Sustainable Development, Central University of Gujarat for providing instrumentation facilities for research study.

## SIGNIFICANCE STATEMENT

The present challenge of soil contamination can be remediated using fodder grass, *Cenchrus ciliaris*. The grass is found efficient in removing heavy metals from the contaminated soil and adapt well to the present experimental condition. The mechanism of heavy metals removal is made efficient by mycorrhizal association. Further researches can be efficiently carried out based on the symbiotic association, adaptation and significant remediation of contaminated sites. *Cenchrus ciliaris* utility as a fodder crop can benefit the farming community after successful field trials. *Cenchrus ciliaris* can be used for conservation of soil moisture, metal removal and utility in farming as the accumulation was found to be more in roots.

## REFERENCES

1. Garbisu, C. and I. Alkorta, 2003. Basic concepts on heavy metal soil bioremediation. Eur. J. Mineral Process. Environ. Prot., 3: 58-66.
2. Ghosh, M. and S.P. Singh, 2005. Comparative uptake and phytoextraction study of soil induced chromium by accumulator and high biomass weed species. Applied Ecol. Environ. Res., 3: 67-79.
3. De Mora, S., S.W. Fowler, E. Wyse and S. Azemard, 2004. Distribution of heavy metals in marine bivalves, fish and coastal sediments in the Gulf and Gulf of Oman. Marine Pollu. Bull., 49: 410-424.
4. Gadd, G.M. and C. White, 1993. Microbial treatment of metal pollution: A working biotechnology? Trends Biotechnol., 11: 353-359.
5. Dubey, K.K. and M.H. Fulekar, 2011. Effect of pesticides on the seed germination of *Cenchrus setigerus* and *Pennisetum pedicellatum* as monocropping and co-cropping system: Implications for rhizospheric bioremediation. Rom Biotechnol. Lett., 16: 5909-5918.
6. Dubey, K.K. and M.H. Fulekar, 2011. Mycorrhizosphere development and management: The role of nutrients, micro-organisms and bio-chemical activities. Agric. Biol. J.N. Am., 2: 315-324.
7. ISTA., 1985. International rules for seed testing. Seed Sci. Technol., 13: 299-355.
8. Yee, D.C., J.A. Maynard and T.K. Wood, 1998. Rhizoremediation of trichloroethylene by a recombinant, root-colonizing *Pseudomonas fluorescens* strain expressing toluene *Ortho*-monooxygenase constitutively. Applied Environ. Microbiol., 64: 112-118.
9. Barman, S.C., R.K. Sahu, S.K. Bhargava and C. Chaterjee, 2000. Distribution of heavy metals in wheat, mustard and weed grown in fields irrigated with industrial effluents. Bull. Environ. Contam. Toxicol., 64: 489-496.
10. Gupta, S., S. Nayek, R.N. Saha and S. Satpati, 2008. Assessment of heavy metal accumulation in macrophyte, agricultural soil and crop plants adjacent to discharge zone of sponge iron factory. Environ. Geol., 55: 731-739.
11. Atlas, R.M. and R. Bartha, 1998. Microbial Ecology: Fundamentals and Applications. 4th Edn., Benjamin Cummings, USA., ISBN-13: 9780805306552.
12. Gadd, G.M., 2004. Microbial influence on metal mobility and application for bioremediation. Geoderma, 122: 109-119.
13. Campbell, R. and M.H. Martin, 1990. Continuous flow fermentation to purify waste water by the removal of cadmium. Water Air Soil Pollut., 50: 397-408.

14. Panchanadikar, V.V. and R.P. Das, 1993. Biorecovery of zinc from industrial effluent using native microflora. Int. J. Environ. Stud., 44: 251-257.
15. Arnebrant, K., E. Baath and A. Nordgren, 1987. Copper tolerance of microfungi isolated from polluted and unpolluted forest soil. Mycologia, 79: 890-895.
16. Huysman, F., W. Verstraete and P.C. Brookes, 1994. Effect of manuring practices and increased copper concentrations on soil microbial populations. Soil Biol. Biochem., 26: 103-110.
17. Smith, S.E. and D.J. Reed, 1997. Mycorrhizal Symbiosis. 2nd Edn., Academic Press, London, pp: 589.
18. Anderson, T.A., E.A. Guthrie and B.T. Walton, 1993. Bioremediation in the rhizosphere. Environ. Sci. Technol., 27: 2630-2636.
19. Rajkumar, M., N. Ae, M.N.V. Prasad and H. Freitas, 2010. Potential of siderophore-producing bacteria for improving heavy metal phytoextraction. Trends Biotechnol., 28: 142-149.
20. Rajkumar, M., M.N.V. Prasad, H. Freitas and N. Ae, 2009. Biotechnological applications of serpentine soil bacteria for phytoremediation of trace metals. Crit. Rev. Biotechnol., 29: 120-130.
21. United States Environmental Protection Agency, 2000. Electro kinetic and phytoremediation *in situ* treatment of metal-contaminated soil: State-of-the-practice. EPA/542. US Environmental Protection Agency, Office of Solid Waste and Emergency Response Technology Innovation Office, Washington, DC., USA.
22. United States Environmental Protection Agency, 2000. Introduction to phytoremediation EPA/600/R-99/107. US Environmental Protection Agency, Office of Research and Development, Cincinnati, OH., USA.
23. Nazir, A., N.R. Malik, M. Ajaib, N. Khan and F.M. Siddiqui, 2011. Hyperaccumulators of heavy metals of industrial areas of Islamabad and Rawalpindi. Pak. J. Bot., 43: 1925-1933.
24. Singariya, P., P. Kumar and K.K. Mourya, 2012. Isolation of some new steroids and evaluation of bio-activity of *Cenchrus ciliaris*. Int. J. Res. Pharm. Sci., 3: 678-684.
25. Khurshid, S., A. Shoaib, A. Javaid and U. Qaisar, 2016. [Fungicidal potential of allelopathic weed cenchrus pennisetiformis on growth of *Fusarium oxysporum* f. sp. *lycopersici* under chromium stress]. Plants Daninha, 34: 453-463.
26. Ines, B.S., A. Muscolo, M. Imed and C. Mohamed, 2016. Effects of irrigations with treated municipal wastewater on phenological parameters of tetraploid *Cenchrus ciliaris* L. J. Food Process Technol., 10.4172/2157-7110.1000553.
27. Keeling, S.M. and G. Werren, 2005. Phytoremediation: The uptake of metals and metalloids by rhodes grass grown on metal contaminated soil. Rem. J., 15: 53-61.
28. Sun, W.H., J.B. Lo, F.M. Robert, C. Ray and C.S. Tang, 2004. Phytoremediation of petroleum hydrocarbons in tropical coastal soils I. Selection of promising woody plants. Environ. Sci. Pollut. Res., 11: 260-266.
29. Agrawal, P., 2007. Ecophysiological and Biochemical studies related to drought adaptation in grass of Indian desert. Ph.D. Thesis, J.N. Vyas University.
30. Bessman, S.P., 1956. Ammonia Metabolism in Animals. In: Symposium on Inorganic Nitrogen Metabolism, McElry, W.D. and B. Glass (Eds.)., The Johns Hopkins Press, Baltimore, Maryland.
31. Qian, J.H., A. Zayed, Y.L. Zhu, M. Yu and N. Terry, 1999. Phytoaccumulation of trace elements by wetland plants: III. Uptake and accumulation of ten trace elements by twelve plant species. J. Environ. Qual., 28: 1448-1455.
32. Muramoto, S. and Y. Oki, 1983. Removal of some heavy metals from polluted water by water hyacinth (*Eichhornia crassipes*). Bull. Environ. Contam. Toxicol., 30: 170-177.
33. Hutchinson, T.C., 1979. Copper Contamination of Ecosystems Caused by Smelter Activities. In: Copper Environment, Niragu, J.O. (Ed.)., 2nd Edn., John Wiley and Sons, New York.
34. Wild, H., 1968. Geobotanical anomalies in rhodesia: 1-The vegetation of copper bearing soils. Kirkia, 7: 1-17.