Research Article

Phytoremediation Efficiency of Sorghum bicolor (L.) Moench in Removing Cadmium, Lead, and Arsenic

Introduction

Wastewater is a new water resource for agriculture and green space. However, wastewater carries pollution of many types, such as heavy metals, synthetic materials and coliform bacteria. Heavy metals are not biodegradable in the environment. When accumulated in ecosystems, heavy metals are very harmful even if present in low concentrations [1].

Soil’s ability to accumulate heavy metals is very important in soil pollution. Deposition of solutes in water or soil may occur at different pH in varying concentrations of many compounds, such as sulfates. This process reduces the soil’s heavy metal content and its rhizosphere concentration through leaching and irrigation runoff [2].

Human activity distributes in the environment numerous toxic heavy metals; this includes industrial and urban activity, as well as agricultural pesticides. Cadmium is a contributoor or the primary agent in toxic heavy metals, because it has a different source for environment disruption and high toxicity, as delineated in numerous research articles [3,4]. Lead is another toxic heavy metal, present in rising proportions in wastewater because it is a component in vehicle fuels, especially in urban settings [5]. Arsenic is yet another heavy metal, possessing a negative ionic charge, leading to differing actions or interactions in soil and root absorption.

Plant remediation/phytoremediation is a leading method for soil decontamination. Plants’ roots, soil and sewage can be treated through degradation, absorption, storage or transfer of polluted reagents [6].

Heavy metal hyper accumulating plants can absorb and accumulate high levels of toxic ions in their tissues, and then can be used as toxic ion remediators. Plants vary in their ability to detoxify different ions in heavy metals from soil and water, so that each hyper accumulator plant may accumulate any heavy metal more than others and be more suitable for any heavy metal ion remediation [2].

Plants used in phytoremediation may produce hazardous biomass with high levels of toxic material, rendering them unusable in food or livestock. Consequently, the selection of plants with high remediation ability and economic value is vital to phytoremediation.

Sorghum is a core agricultural plant in tropical areas. It is the fifth most consumed cereal in the world, used as food, animal feed and biofuel [7]. Sorghum can grow through wastewater irrigation, and has many remediation properties in soil pollution [8].

The aim of this research is to investigate the practical remediation ability of sorghum on heavy metal–polluted wastewater to improve an actual method for sewage usage.

Material and Methods

Culture conditions

3–5 seeds of sorghum were cultivated in pots 30 cm high (15 kg) in 16 h light and 8 h dark photoperiods, at 28 °C and 18 °C, respectively. The pots were irrigated 3 times a week during a 12-week growth period.

Soil analysis

Soil samples were taken from all treated pots (100 gr),...
homogenized and used for analysis after cultivation. Soil analysis comprised base soil analysis under standard methods (Table 1).

**Wastewater treatment**

1500 l municipal wastewater was disinfected by activated oxygen, and stored in a cool and dark condition during growth periods. Different organic, salt and ionic parameters of wastewater were analyzed by standard methods (Table 2). 1 gr of cadmium chloride, lead nitrate or sodium arsenate was added to 1 l distilled water. 1 ml of each stock was added to 100 ml of municipal wastewater and produced the treatment, “10 mg”. 10 ml of this solution was added to 100 ml of wastewater and produced the treatment called, “1 mg”. 10 ml of this solution was added to 100 ml of wastewater to produce the treatment named, “0.1 mg”. Each treatment was immediately used in irrigation.

**Acidic digestion**

Plants undergoing cadmium and lead treatment were collected and divided into root, stem, leaf and seed portions. The samples were dried at 80 °C and heated in a furnace set at 550 °C for 5 h. 100 mg of the ash was mixed with 5 ml HCL 2 normal and heated in 80 °C for 2 h after resting for one night. In the arsenic treatment, 100 mg of ash was mixed with 2 ml of concentrated H2O, and heated to 80 °C for 1 h, then mixed with 3 ml concentrated HNO3 and rested one night.

Soil samples from each treatment were powdered after drying and heated at 550 °C for 5 h. 1 gr of soil was mixed with 3 ml HCL 2 normal and 3 ml concentrated HNO3, then heated at 80 °C for 1 h and rested one night. All samples were filtered and injected into an atomic absorption set, Varian AA240 FLAME a model.

**Absorption coefficient**

Absorption coefficient (AC) is a measure of the ability of plant or soil to accumulate heavy metals or ions, or to accumulate in a specific site. This can be calculated by dividing the ion concentration of two samples, such as plant and soil. For example, the AC of plant on soil was calculated by Eq. (1):

Equation 1:

\[
\text{Absorption Coefficient (AC)} = \frac{\text{plant heavy metal content (ppm / DAW)}}{\text{soil heavy metal content (ppm / DW)}}
\]

Table 1: Soil parameters.

| No. | Parameter            | Value | Dimension |
|-----|----------------------|-------|-----------|
| 1   | Texture              | Loam  |           |
| 2   | Clay                 | 19    | %         |
| 3   | Silt                 | 30    | %         |
| 4   | Sand                 | 51    | %         |
| 5   | Moisture (SP)        | 45    | %         |
| 6   | Electrical exchange (EC) | 13.6 | ds/m     |
| 7   | pH                   | 7.9   |           |
| 8   | Total neutralizing value | 21.5 | %         |
| 9   | Organic Carbon (O.C.) | 1.3  | %         |

Table 2: Wastewater parameters. The analysis shows that pH and ionic concentration are high.

| No. | Parameter            | Value | Dimension |
|-----|----------------------|-------|-----------|
| 10  | Water hardness (CaCO3) | 832   | ppm       |
| 11  | Ca                   | 128   | ppm       |
| 12  | CL                   | 700   | ppm       |
| 13  | Total alkalinity (CaCO3) | 323   | ppm       |
| 14  | Mg                   | 122   | ppm       |
| 15  | SO4                  | 670   | ppm       |
| 16  | NO3                  | 5.7   | ppm       |
| 17  | NO2                  | 0.06  | ppm       |
| 18  | NH4                  | 28    | ppm       |
| 19  | PO4                  | 2.2   | ppm       |
| 20  | EC                   | 3930  | µs/cm     |
| 21  | Salts                | 2     | %         |
| 22  | Na                   | 520   | ppm       |
| 23  | K                    | 18    | ppm       |
| 24  | COD                  | 55    | ppm       |
| 25  | BOD5                 | 21.3  | ppm       |
| 26  | Turb                 | 4     | NTU       |
| 27  | TDS                  | 2080  | mg/litre  |
| 28  | Temperature          | 20    | °C        |

AC of irrigated soil, irrigated plant and plant in soil were calculated. The AC of different heavy metals in plant was compared per Eq. (2) (Table 6):

Equation 2:

\[
\text{two ions AC comparison} = \frac{\text{plant heavy metal A content (ppm / DAW)}}{\text{soil heavy metal A content (ppm/ DW)}} - \frac{\text{plant heavy metal B content (ppm / DAW)}}{\text{soil heavy metal B content (ppm / DW)}}
\]

**Statistical analysis**

SPSS 20 software (SAS Corp., Chicago, USA) was used in statistical analysis. Normal distributions of data, independence or randomness, were tested by the Kolmogorov-Smirnov test (1-sample K-S), and the RUN TEST, respectively. Comparison of meaning in different treatments was calculated by One-Way ANOVA and LSD as post hoc option. Multivariate analysis utilized the ANOVA test.

**Results**

**Plant heavy metal accumulation**

Three treatments of irrigation wastewater with 10, 1 and 0.1 ppm cadmium chloride with three repeats were used in the cadmium treatment. Plant cadmium content increased with wastewater cadmium concentration (Table 3). One-way variance analysis (ANOVA) demonstrated that the difference of all groups was significance (P < 0.5).

Lead remediation was analyzed by three concentrations of lead acetate in wastewater. No variation was observed among the three groups of treatment based on ANOVA analysis. On the other hand,
when the concentration of lead increased 100 times in irrigation, no change occurred in plant lead accumulation (Table 3).

LSD analysis of three concentrations of sodium arsenate in plant showed that the 1 ppm and 10 ppm treatments had higher arsenic levels than 0.1 ppm treatment ($P < 0.05$). On the other hand, when arsenic concentration increased in irrigation, plant arsenic rose only within a limited range (Table 3).

Comparison among the three heavy metals’ accumulation in sorghum yielded the following order: cadmium > lead > arsenic in high heavy metal concentration in irrigation (10 ppm); however, in low concentration (0.1) the order changes to lead > cadmium > arsenic.

**Soil heavy metal accumulation**

Since heavy metals are imported with irrigation to farm or agriculture, understanding the extent of the soil’s heavy metal accumulation ability is imperative. Table 3 shows the heavy metal content of soil and its variation between different treatments. Although heavy metal concentrations change 100 times in irrigation, soil heavy metal content does not vary in arsenic and is very low in lead ($10.5 \pm 1.7$ ppm). In cadmium, with an increase in heavy metals in irrigation, soil can accumulate cadmium in high concentrations, shown in a saturated curve (Figure 2).

3**Tissue treatment**

Root, stem, leaf and seed of plant were separated and analyzed based on dry ash weight (DAW) and dry weight (DW) of tissue. The cadmium and lead content of all tissues in DAW were equal, but this was not the case with arsenic. Roots accumulate arsenic more than other tissues, so that root has more accumulators and the stem accumulates fewer ions than other tissues, in the following order: root $\geq$ leaf $\geq$ stem $=$ seed. All three ions had the same pattern (Table 4).

**Runoff**

To investigate the likelihood of deep soil or groundwater pollution through irrigation, pots that had been irrigated by 10 ppm heavy metals during the growth period were leaching out by untreated wastewater at the end of the experiments. The runoff was collected and analyzed in three repeats. Results show, although the soil concentration in cadmium treatment is higher than that of lead and arsenic, its concentration in runoff was below 1 ppm (Table 5).

**Absorption coefficient**

The remediation of water and soil by soil and plant were calculated by absorption coefficient parameter. Results showed that soil could remediate heavy metals of wastewater in low concentration. Plant AC was very high for lead and cadmium based on irrigation (1136 and 750, respectively). Although plant can concentrate these toxic ions from 2–18 times in its soil concentration based on DAW, these ability is greater in low concentration. Consequently, the absorption

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**Table 3:** Soil and plant heavy metal accumulation in sorghum.

| Irrigation (ppm) | Soil (ppm/ DW) | Plant (ppm/ DAW) |
|-----------------|---------------|-----------------|
|                 | Cadmium*      | Lead*           |Arsenic| Cadmium* | Lead | Arsenic* |
| 10              | 123 ± 5       | 12.5 ± 0.5      | 6.6 ± 1.5 | 238 ± 33 | 183 ± 43 | 18 ± 7   |
| 1               | 37 ± 2        | 10.5 ± 0.5      | 6.3 ± 1.5 | 123 ± 28 | 180 ± 39 | 17 ± 5   |
| 0.1             | 5 ± 1         | 8.5 ± 0.5       | 4.8 ± 2.2 | 75 ± 5   | 117 ± 29 | 1.6 ± 1.3|
| Control         | 0             | 0.63±0.15       | 0.47±0.09 | 0        | 19 ± 2.6 | 0.66±0.05|
| Total           | 55 ± 52       | 10.5 ± 1.7      | 5.9 ± 1.7 | 145 ± 75 | 160 ± 46 | 12.5 ± 9 |

* The difference of means between groups (10, 1, 0.1 and control) of this column is significant at the 0.05 level.
The increase in soil and plant cadmium content can be plotted in a logarithmic curve from 0.1 to 10 ppm of treatment in wastewater (data not shown), as reported by researchers [14]. The lead content of soil and plant did not increase and had poor correlation with lead irrigation concentrations. Most concentration observed in 0.1 treatments was comparable with cadmium treatment. Moreover, when lead concentration changed 100 times in irrigation, soil content changed 0.3 times ($y = 0.868\ln(x) + 10.5$, $R^2 = 0.941$), and then the plants’ concentration of lead in different treatment is the same ($P>0.05$). Since lead is detectable in runoff despite cadmium (Table 6), it seems that as it exited the pots, and the results were not compatible with many researchers’ reports that lead can concentrate in first level of soil without being diffused to groundwater [15]. Similar to lead, soil arsenic is constant in different treatments and is detectable in runoff, although this runs opposite to other researchers’ reports; soil arsenic concentrations increase with changes to irrigation arsenic content [16]. Since the solution properties or sensitivity of cadmium, lead or arsenic differs in water or soil, such differences may relate to many other parameters of soil or wastewater, such as sulfate and pH (Tables 1, 2) [17]. This can result in the dissolution or precipitation of these ions when added to wastewater; this can be observed in lead by examining the changes in the transparency and shininess of wastewater. Then the dissolution of ions reduces ion interaction and activity with soil particles and exits the soil by leaching.

This result demonstrates that the wastewater has the definite heavy metal capacity based on its chemical properties, and may be managed for agricultural purposes by limiting heavy metal concentration.

Tissue analysis of sorghum based on DAW showed that the content of cadmium or lead is equal in all parts of plants and that ions distribute normally in all tissues or organs. However, these results do not appear based on DW as reported by previous researchers. Based on DW, the cadmium and lead are distributed in different tissues in this order: root > or = leaf > or = stem > seed. Since stem and seed in sorghum are the carbon storage organs [18], results show that the storage of heavy metals in sorghum is related to ash or macro elements content, but not to carbohydrate or tissue type. The different tissues had the same power in heavy metals transport or storage; previous reports in variations between organs may relate to consideration of storage molecules in the cell. These results change many previous ideas in decreasing the transportation of cadmium from root to shoot or other organs [19-21], because most of them calculate cadmium concentration based on DW, the carbon storage of plant may reproduce any mistake in this process.

Previous research shows the distribution of arsenic in agricultural plants such as onion, cauliflower, rice, brinjal and potato found in the following order: roots > stem > leaves > seed or edible parts [22], but this order in sorghum is in fact root > leaf = seed = stem based on DW. As previously mentioned, in cadmium and lead, stem of sorghum is a carbohydrate or storage part of the plant. This result confirms that heavy metal accumulation does not result in any change in coloration with carbohydrate stored in its physiological state.

In sorghum, root accumulates more arsenic than other tissues, based on DAW. This result has been reported by previous researchers.

### Discussion

Wastewater is a new source of irritation, but carries pollutants such as heavy metals that cannot be degraded in the environment. Phytoextraction is a biological method for cleanup of heavy metal polluted soils. This process is a feature of many plants, so that many researchers have suggested that plants can accumulate rare heavy metals such as cadmium up to 0.01% DW as phytoremediator or hyper accumulator [9]. Numerous plants were identified as hyper accumulators but most of them are not used in agricultural or industrial applications, or are harmful to health after cultivation in polluted soil [10,11]. Since this method is both costly and produces polluted waste, the selection of plants is very important. In this research, we chose to work with sorghum, which can absorb various heavy metals and has different usages in food or bioethanol production [12]. With this information, we know that the polluted plant after remediation can be used in bioenergy.

The ability of soil to accumulate heavy metals is very important in soil pollution remediation and plant heavy metal uptake. This is related to soil properties and compounds [13]. In this research, lead and arsenic soil content was constant when concentration of treatment increased in irrigation, but in cadmium the soil can increase its heavy metal content parallel with treatment (Figure 1). Subsequently, the heavy metal content of soil had significant variation and is a parameter in plant heavy metal accumulation and its toxicity (Table 3).

### Table 4: Tissues’ heavy metal content in DAW and DW.

| Tissues | Cadmium | Lead | Arsenic |
|---------|---------|------|---------|
|         | DAW     | DW * | DAW     | DW * |
| Root    | 241 ± 67 | 90 ± 34 | 195 ± 73 | 78 ± 29 |
| Stem    | 225 ± 65 | 4.7 ± 1 | 143 ± 49 | 2.5 ± 0.9 |
| Leaf    | 240 ± 51 | 42 ± 2 | 274 ± 70 | 54 ± 14 |
| Seed    | 250 ± 56 | 17 ± 3.4 | 170 ± 139 | 26 ± 20 |
| Average | 239 ± 52 | 38 ± 37 | 195 ± 92 | 40 ± 33 |

*: not detectable (<0.1 ppm)

This result shows AC displays variation based on heavy metal type and its concentration.

### Table 5: Runoff collected after cultivation at 10 ppm.

| Treatment | Soil (ppm) | Runoff (ppm) |
|-----------|------------|--------------|
| Cadmium   | 123 ± 5    | 0.00*        |
| Lead      | 8.5 ± 0.5  | 5.8 ± 0.76   |
| Arsenic   | 4.8 ± 2    | 4 ± 2.6      |

*: not detectable (<0.1 ppm)
for other agricultural plants [23]. This refers to excess or different mechanisms of arsenic storage in root compared with other tissues and other ions (cadmium and lead). These results show different root-shoot heavy metal pattern distribution between arsenic and cadmium or lead, although the main pattern among the four tissues in three ions is the same based on DW in the following order: root > or = leaf > or = stem = seed, and is related to hydrocarbon storage.

Many researchers revealed that in many plants, such as Oryza sativa, arsenic produces a thin plaque out of rhizoderm of root with Fe hydroxide as a cover [24]. This cover reduces arsenic absorption by the root and increases local arsenic content of root (apoplastic arsenic) compared with other tissue. Similarly, sorghum’s low ability to accumulate arsenic in the same soil concentration compared to lead and cadmium and the high content of root arsenic compared to other tissue in DAW may be related to this phenomenon.

In soil with constant arsenic, the AC or plant/soil arsenic uptake increases when arsenic in irrigation change that not observed in cadmium or lead. However, this difference may relate to direct adsorption of arsenic from wastewater as a plaque on the surface of root as mentioned by many researchers [24], before exchange with soil or exiting rhizosphere will occur as runoff.

Bioaccumulation ability of heavy metals

Results show the AC or bioaccumulation ability of heavy metals in sorghum changes through many factors, such as the types of ions and their concentration. Therefore, in low concentration of heavy metals in irrigation, AC is ordered as follows: cadmium = lead > arsenic (Table 6).

Based on irrigation treatment, the soil and plant AC equation curve descended in cadmium, lead and arsenic (data not shown). These equations showed that bioaccumulation rate or ability was inversely related to ion concentration (Table 6), as mentioned by many researchers [25]. On the other hand, the most effective phytoremediation takes place in low concentrations, because when high coefficient absorption for soil and plant was shown in this range, groundwater was more protected.

Since lead and cadmium content is related to DAW in all tissues (Table 4), it may be suggested that these ions uptake with cationic and nonspecific or common transporters in plant. This finding shows the similarity of cadmium and lead transporters, as reported by many previous researchers. This suggests that lead may uptake by nonspecific cation channel related to very high potential membrane [26], and low affinity protein transporters can mediate K+, Na+, Ca+, lead and cadmium uptake [27].

Moreover, the descending saturation curve of cadmium AC or plant/soil absorption in various irrigation differs from diffusion or a non-energetic transportation curve (Figure 3). Therefore, cadmium needs a carrier or protein active site for transport across membranes, and accumulation in plant with high efficiency in low concentration.

Conclusion

This research demonstrates that with low pollution in municipal wastewater, sorghum is a good plant for remediation and can absorb high levels of cadmium and lead, so that with no cultivation these ions can accumulate in toxic level in soil or leak deep into soil or groundwater. However, the harvested plant can accumulate a high level of heavy metals, which then must be used as non- foods or livestock such as bio energy.

The variation of absorption coefficient of cadmium and lead and their tissue accumulation patterns was differ from arsenic in sorghum so that each heavy metal has a distinct behavior base on its properties.
In high levels of pollution, soil accumulates high levels of cadmium and the toxicity of soil increases with less groundwater pollution compared to lead and arsenic, as reported by many researchers.

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