



BIOACCUMULATION OF As AND Pb IN POT MARIGOLD GROWN IN SEWAGE IRRIGATED SOILS OF PRAYAGRAJ, UTTAR PRADESH, INDIA

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Abstract

A pot experiment was conducted to observe the bioaccumulation of Arsenic (As) and Pb in *Calendula officinalis* L. (ornamental plant) grown in sewage irrigated soils of Prayagraj. Four different sewage-discharge points (situated at Naini, BaluaGhat, Daraganj and Mumfordganj regions of Prayagraj) were selected for collecting sewage samples. The study revealed that different sewage-discharge points showed enrichment and bioaccumulation of heavy metals (As and Pb) in the soils and plants, respectively, in proportion with the degree of pollution or levels of heavy metals applied through the sewage water irrigation. Soils contained a detectable amount of arsenic (0.005-0.08 mg kg⁻¹, mostly below the permissible limit) and a higher amount of Pb (6.00-7.52 mg kg⁻¹), particularly in sewage-irrigated pots. Arsenic (As) content was found 0.05-0.20 mg kg⁻¹ and Pb ranged from 2.82-7.50 mg kg⁻¹ in the plants grown in sewage-irrigated soil. The study concluded that *Calendula* crop grown in sewage irrigated soils accumulated As and Pb which pose a potential for phytoremediation of As and Pb in the sewage irrigated soils.

Key words: Arsenic, Bioaccumulation, Lead, Sewage water, Phytoremediation

Introduction

The problem of environmental pollution is increasing day by day duets rapid industrialization and urbanization. Besides thus, anthropological activities have put an increasing burden on the environment by releasing large quantities of hazardous wastes heavy metals (Cd, Cr, Pb) and various organic contaminants. The enrichment of heavy metals and metalloids in soils continues to create serious clinical problems, as these metals and metalloids cannot be degraded into non-toxic forms but persist in the ecosystem. (Asgari *et al.*, 2018) Environmental pollution through heavy metals is a serious problem in most countries around the globe, which may be caused by natural processes and anthropogenic activity. This finding provides a comprehensive view and also the severity of soil heavy metals pollution all over the world. In other words, long-term exposure of soil heavy metals (toxic metals) has mainly caused acute threat to environment pollution and also harm to human health, since heavy metals are essentially engrossed by human body through three potential pathways such as ingestion,

inhalation, and dermal contacts (Adimalla, 2019, Hu *et al.*, 2017, Krishna & Mohan 2016, Liu *et al.*, 2018, Pan *et al.*, 2018, Zhaoyong *et al.*, 2018, 2019).

Arsenic, a toxic trace metalloid, is of great environmental concern due to its presence in soil, water, and plant, animal and human continuum. Its high toxicity and increased appearance in the biosphere have triggered public and political concern. Out of 20 countries (covering Argentina, Chile, Finland, Hungary, Mexico, Nepal, Taiwan, Bangladesh, India, and others) in different parts of the world, where groundwater arsenic contamination and human suffering there from have been reported so far, the magnitude is considered to be the highest in Bangladesh, followed by West Bengal, India (Sanyal *et al.*, 2015). Arsenic is a widely occurring toxic metalloid in natural ecosystems. As small as 0.1 g of arsenic trioxide can prove lethal to humans (Jarup, 1992).

The sources of heavy metals in the surface environment are natural and anthropogenic. Natural sources include parent rocks and metallic minerals. Anthropogenic sources include agriculture (fertilizers, pesticides, etc.), metallurgy (mining, smelting etc.), energy

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production (power plant, leaded gasoline, etc.) and sewage disposal (Navratil & Minaril, 2005, Odika *et al.*, 2020). The most of the heavy metals are toxic at low concentrations and are capable of entering the food chain, where they accurate and cause damage to living organisms. Almost all metals have the potential to exhibit harmful effects at higher concentrations and the toxicity of each metal depends on the amount available to organisms, the absorbed dose, the route and the duration of exposure. The results of a principal components analysis showed that Cr, As, and Ni are mainly released by weathered rocks, whereas metallic contaminants such as Hg and Pb are generated by industries, vehicular fumes, and PM, and wastewater reuse for irrigation (Liu *et al.*, 2011a, 2011b). The identification of soil contaminants and their sources is essential to research because of their tight links to human health (Velea *et al.*, 2009, Khan *et al.*, 2010, Rai, 2012). Children are especially at greater risk because they have higher intestinal Pb absorption causing more vulnerable nervous systems that are still under development (Ahamed & Siddiqui, 2007, Zhai *et al.*, 2015). It inhibits various biochemical and physiological activities (Kosobrukhov *et al.*, 2004) causing visible symptoms of toxicity leading to a reduction in biomass and growth of plants (Rossato *et al.*, 2012).

The toxicity and bioaccumulation tendency of heavy metals in the environment is a serious threat to the health of living organisms. Unlike organic contaminants, heavy metals cannot be broken down by chemical or biological processes and they can only be transformed into less toxic species. Plants are deferred in their ability to accumulate heavy metals (Nouri *et al.*, 2009, Kacalkova *et al.*, 2015) for this concern, the selection of plant species for phytoextraction of heavy metals depends mainly on the ability of tolerant capacity and the biomass of the selected plant (Rezania *et al.*, 2016). The high biomass of plant the high metal ions removed from the treated soil. Some plant species can accumulate high contents of heavy metals in their tissues; however, they produce little biomass and are slow-growing plants, which makes it infeasible to use these species in phytoremediation. Thus, plant species have a dilemma with respect to balancing the uptake and maintaining concentrations of heavy metals in their tissues to cope with heavy metal stress. Nevertheless, metal-tolerant plants are either grouped as metal accumulators or metal excluder based on the bioaccumulation of metals in their roots or aerial tissues, respectively (Mani *et al.*, 2012). The pot marigold (*Calendula officinalis* L.) is an important medicinal and ornamental plant with numerous pharmaceutical and

industrial applications. It plants display antimicrobial, antispasmodic, antitumor and antioxidant activities due to many active components, mainly flavonoids (Ukiya *et al.*, 2006). The selected *Calendula officinalis* L. for phytoremediation of heavy metals depends mainly on the ability of accumulated capacity of the plant. The selected this crop of mine means that it should also improve the heavy metals from the soil; it should not have any effect in our people. Due to the harmful effects of these heavy metals, there are growing environmental and public health concerns, and need of increasing awareness in order to remediate the contaminated soils.

An estimated 12.6 million people have died worldwide in recent years from >100 diseases caused by unhealthy environments such as contaminated soils (WHO, 2016). During the past decade, As poisoning and resulting adverse health effects have been reported in Bangladesh. Among that nation's population of 125 million, 35-77 million people have been affected by As poisoning, making it the largest known mass poisoning by contaminants in human history (Shakoor *et al.*, 2019, Smith *et al.*, 2000). Based on 2015 data, the Institute for Health Metrics and Evaluation and the World Health Organization (WHO) have reported 494, 550 deaths and a loss of 9.3 million disability-adjusted life years resulting from long-term Pb exposure of humans (WHO, 2018). Young children are particularly susceptible to Pb poisoning due to the ingestion of Pb-contaminated soil or dust, and many young children in Nigeria, Senegal, and many other countries have died from exposure to Pb-contaminated soil (Keller *et al.*, 2017, WHO, 2018).

This study was conducted to assess the depth-wise distribution, of As and Pb bioaccumulated of heavy metals (As and Pb) by some pot marigold (*Calendula officinalis* L.) ornamental plant, the main objectives (1) to overcome the problems associated with As and Pb toxicity and sewage irrigated soils and bioaccumulated of heavy metals (As and Pb) by growing pot marigold (*Calendula officinalis* L.) ornamental plant. (2) To observe the enrichment and bioaccumulation of heavy metals (As and Pb) in the sewage-irrigated soils and plants, at different sites of the Prayagraj region. (3) To check the potential of *Calendula* crop for phytoremediation of As and Pb in the sewage irrigated soils.

Materials and Methods

Experimental site

The experimental site is situated in Northern India at 24R⁰58'N latitude and 80R⁰56'E longitude on south-east facing slopes of comparable inclination at altitudes between 170 and 85 m above sea level. A sand clay loam

soil, derived from sewage-sludge irrigated Indo-Gangetic alluvial soils of Sheila Dhar Institute of Soil Science farm situated on the confluence of Ganga and Yamuna alluvial deposit, was sampled from four different sewage-discharge points (situated at Naini, BaluaGhat, Mumfordganj and Daraganj regions of Prayagraj) were selected for collecting sewage samples. The properties of the soil were: pH 7.76 ± 0.36 , EC (dSm^{-1}) 0.27 ± 0.07 , organic carbon (%) 0.56 ± 0.02 , total N (%) 0.07 ± 0.01 , total P (%) 0.08 ± 0.01 , CEC (C mol (P) kg^{-1}) 19.7 ± 0.15 . The texture comprised of sand ($>0.2\text{mm}$) $55.0 \pm 3.61\%$, silt ($0.002\text{-}0.2\text{mm}$) $21.0 \pm 4.58\%$ and clay ($<0.002\text{mm}$) $24.0 \pm 4.35\%$.

Soil sampling and extraction of heavy metals from soil

Soil samples collected from different discharge points of Naini, BaluaGhat, Mumfordganj and Daraganj. In each sampling unit, soil samples were drawn from several spots in a zigzag pattern leaving about 2 m area along the field margins. Silt and clay were separated by the Pipette method and fine sand by decantation. For total As and Pb content, 2 gram of soil was mixed in 5 ml of HNO_3 (16M, 71%) and 5ml of HClO_4 (11M, 71%). The composite was heated up to dryness. The volume was made up to 50 ml with hot distilled water. The clean filtrate was used for the estimation of the heavy metals (As and Pb) by Atomic Absorption Spectrophotometer (AAS) (AAnalyst600, Perkin Elmer Inc., MA, USA).

Soil physico-chemical analysis

Soil pH was measured with a 1:2.5 soil-water ratio using an electrical digital pH meter. Double distilled water was used for the preparation by chromic acid digestion method, cation exchange capacity (CEC) by neutral 1 N ammonium acetate solution, total nitrogen by digestion mixture (containing sulphuric acid) selenium dioxide and salicylic acid) using micro-Kjeldahl method, Glass Agencies, Ambala, India (Kumar and Mani 2010) and total phosphorus by hot plate digestion with HNO_3 (16M, 71%) and extraction by standard ammonium molybdate solution (Chopra and Kanwar, 1999; Kumar and Mani 2010).

Plant analysis

Plants were harvested after 60 days (harvesting stage). Samples were carefully rinsed with sewage-irrigated water followed by 0.2% detergent solution, 0.1N HCl, de-ionized water, and double-distilled water. Later samples were dried in a hot-air oven at a temperature of 45°C and ground to a fine powder. Plant dry biomass weight was recorded. One gram of ground plant material was digested with 15ml of the tri-acid mixture (Kumar

and Mani, 2010) containing conc. HNO_3 (16M, 71%), H_2SO_4 (18M, 96%) and HClO_4 (11M, 71%) in 5:1:2), heated on hot plate at low heat (60°C) for 30 minutes and total heavy metals were determined by the aforesaid Spectrophotometer.

Bioaccumulation factor and translocation factor

Bioaccumulation factor (BF), defined as the ratio of chemical concentration in a plant (root and shoot tissues) to soil, is used to measure the effectiveness of a plant in concentrating pollutant into aerial part (Fayiga *et al.*, 2004), and translocation factor (TF), the quotient of contaminant concentration in shoots to roots, which is used to measure the effectiveness of a plant in transferring a chemical from roots to shoots (Sun *et al.*, 2011). Bioaccumulation factor (BFs) is calculated according to the following formula.

$$\text{BFs} = \frac{M_{\text{shoot}}}{M_{\text{soil}}}$$

Where, M_{shoot} is the metal content (mg kg^{-1}) in shoots, M_{soil} is the total metal content (mg kg^{-1}) in the soil. M_{soil} was calculated by found in total metal content in soil naturally with founded metal content in the sewage-irrigated soil. Translocation factor (TFs) is calculated according to the following formula:

$$\text{TFs} = \frac{M_{\text{shoot}}}{M_{\text{root}}}$$

Where, M_{root} is the metal concentration in roots of the plants (mg kg^{-1}).

Statistical Analysis

The experiment results were expressed as mean \pm standard error of mean (SEM) of three replicates. GraphPad prism 8 software was using for drawing Figures.

Results and Discussion

Concentration of As and Pb in sewage irrigated soil

The depth-wise distribution of sewage-irrigated soil concentration of As and Pb was sewage irrigated soil at all the four site depth (in cm.) 0-15, 15-30, 30-40 and 40-60. The application of As and Pb found in the concentration sewage irrigation of soils in site Naini are highest $0.20\text{-}0.05 \text{ mg kg}^{-1}$ and $7.50\text{-}3.12 \text{ mg kg}^{-1}$, BaluaGhat As $0.17\text{-}0.04 \text{ mg kg}^{-1}$, Pb $7.12\text{-}3.08 \text{ mg kg}^{-1}$, Daraganj in As $0.15\text{-}0.02 \text{ mg kg}^{-1}$, Pb $6.84\text{-}2.82 \text{ mg kg}^{-1}$ and lowest in Mumfordganj As $0.13\text{-}0.01 \text{ mg kg}^{-1}$, Pb $6.26\text{-}2.76 \text{ mg kg}^{-1}$. The among study was Naini profile having to fund comparatively higher sewage-sludge are characterized by the higher heavy metal contents as compared to the other sewage region of Prayagraj, because which resulted in the available As and Pb of the sewage irrigated soils, the higher build-up of Pb was

because of a higher level of air pollutants. The increasing trend of Pb due to the exhausts of vehicles (Adamiec *et al.*, 2016), and the As contending increased due to the discharge of industrial waste (*i.e.* dye industries, tanneries, batteries) and insecticide Fig. 1, 3. (Adimalla & Wang 2018, Giri & Singh 2017, Krishna & Mohan 2016, Machender *et al.*, 2011; Machender *et al.*, 2013) the reported that highly concentrations of As in Uttar Pradesh, Jharkhand and Telangana is due to industrial activities and anthropogenic sources of pesticides. Leaching and transportation of As from topsails to the underlying soils represent a possible pathway of As removal from the top soils (Farooq *et al.*, 2010, Polizzotto *et al.*, 2006). That have As accumulated in the top few centimeters, during the monsoon flooding, diffuses into the floodwater, and thus, As concentration in the topsails remains lower than the expected values (Dittmar *et al.*, 2007).

Bioaccumulation of As and Pb grown in *Calendula officinalis* L.

The available heavy metals in four sites of the Prayagraj region in the sewage irrigated soils were As and Pb Fig. 1, 2. The pot marigold (*Calendula officinalis* L.) grown in heavy metal-enriched sewage irrigated soil took up metal ions in varying degrees. The heavy metal bioaccumulation have highly accumulated As 74.67% and

Pb 78.60% founded in *Calendula officinalis* L. crop in Naini region, the found in edible part in *Calendula* crop respectively, root (As and Pb 0.08 ± 0.04 , 3.21 ± 0.12 mg kg⁻¹) were observe higher bioaccumulation heavy metal (As and Pb) as compare with shoot (As and Pb 0.06 ± 0.04 , 2.76 ± 0.14 mg. kg⁻¹). Which are the lowest accumulation of the heavy metals contaminated sewage-irrigated soil in Mumfordganj region grown in pot marigold was found in As 66.36% and Pb 69.21%, the found in the edible part root (As and Pb 0.05 ± 0.03 , 2.67 ± 0.14 mg kg⁻¹) and shoot (As and Pb 0.03 ± 0.02 , 1.66 ± 0.09 mg kg⁻¹). Which are the BaluGhat, Daraganj region grown in *C. officinalis* was bioaccumulation found in As 72.53%, 68.23% and Pb 76.24%, 75.56%. The growing of Pot Marigold plants in sewage irrigated soils it shows all sites bioaccumulation As and Pb in roots (average As 0.08-0.05 mg kg⁻¹, Pb 3.21-2.67 mg kg⁻¹) in comparison to shoots (average As 0.06-0.03 mg kg⁻¹, Pb 2.76-1.66 mg kg⁻¹) in all sites of experiments performance of *Calendula officinalis* L. for As and Pb in plant growth to make the best use of the phytoremediation efficiency of heavy metals. Among the tested crop pot marigold (*Calendula officinalis* L.), the order of accumulation of heavy metals was Root > shoot Fig. 2, 4. Growing

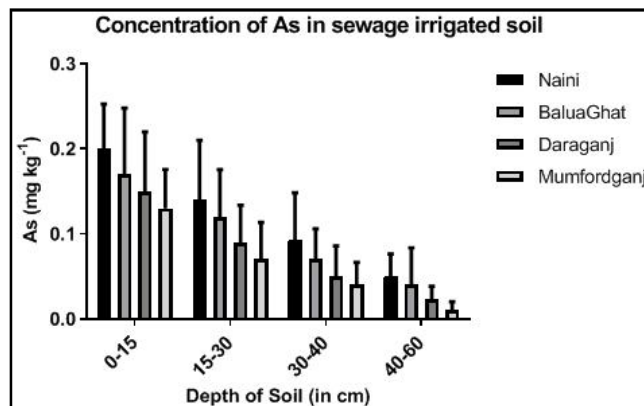


Fig. 1: Concentration of As in sewage irrigated soil.

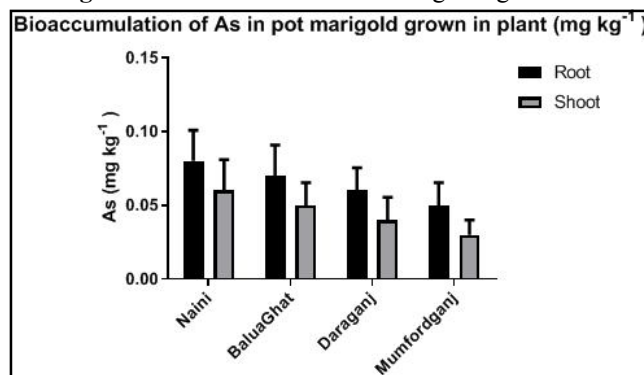


Fig. 2: Bioaccumulation of As in *Calendula officinalis* L. grown in plant (Mg kg⁻¹).

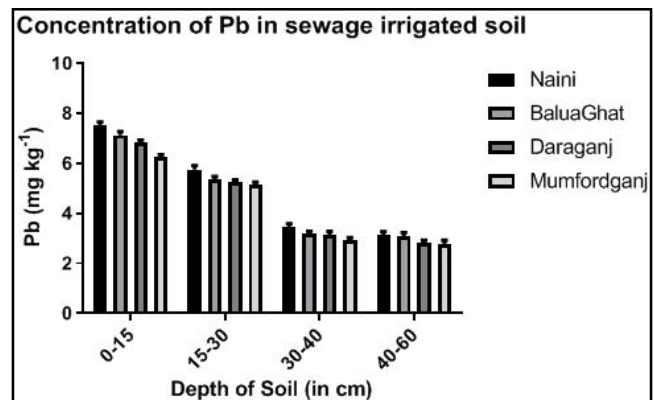


Fig. 3: Concentration of Pb in sewage irrigated soil.

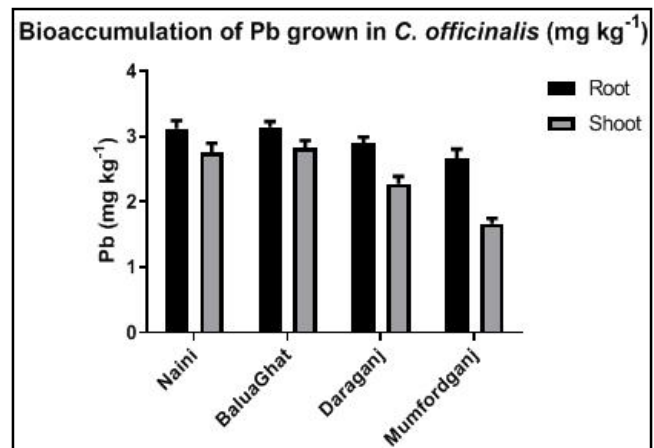


Fig. 4: Bioaccumulation of Pb grown in *Calendula officinalis* L. (mg kg⁻¹).

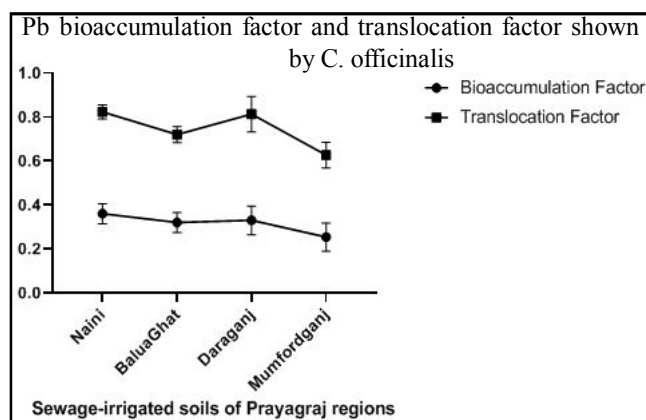


Fig. 5: Pb bioaccumulation factor and translocation factor shown by *C. officinalis* from sewage irrigated soils of Prayagraj regions.

Table 1: Pb bioaccumulation factor and translocation factor shown by *C. officinalis* from sewage irrigated soils of Prayagraj regions.

| Sites (Prayagraj regions) | Bioaccumulation Factor | Translocation Factor |
|---------------------------|------------------------|----------------------|
| Naini | 0.36±0.02 | 0.85±0.03 |
| BaluaGhat | 0.32±0.04 | 0.72±0.02 |
| Daraganj | 0.33±0.06 | 0.78±0.05 |
| Mumfordganj | 0.26±0.01 | 0.62±0.01 |

biomass with the potential for metal uptake needs further investigation, with the aim of exploring the metabolic potential of this growing biomass and their application in contaminated soil management. Heavy metals are transported from the root to the aerial part of the plants by xylem loading, whereas foliar transport involves the phloem vascular system. Concerning the mechanisms of heavy metal uptake in the crops, foliar translocation has been studied less than the root accumulates mechanism (Shahid *et al.*, 2016).

Bioaccumulation factor and translocation factor

The bioaccumulation factor and translocation factor values have been illustrated under the table 1, Fig. 5. The ability to move or be moved freely and easily of Pb from soil solution to plant tissues was enhanced by the found in all sites of sewage-irrigated soils in the Prayagraj regions of Pb from 2.76-7.50 mg kg⁻¹ Fig. 3.

The ability of the growing pot marigold (*Calendula officinalis* L.) plant for Pb ions accumulated. The value of bioaccumulation factor was observed that high concentration found in sewage soils in the plant with increased concentrations of Pb presented higher bioaccumulation factor 0.36, the obtained result showed that *Calendula officinalis* L. maybe use effectively for the removal of Pb contaminated soils. Shows the higher translocation factor 0.85 of Pb was observed at the higher

concentrations in the root and shoot (in mg kg⁻¹). However, this decrease when lead accumulation increase from shows that the higher accumulation plant shoots tissues restricted more shoots Pb bioaccumulation table 1, Fig. 5. The maximum translocation factor 0.86.

Conclusion

Among the four sites of experiments of bioaccumulation Naini site having the highest concentration of As and Pb in sewage-irrigated soils because of heavy metals content sewage-irrigated water discharge from Naini industrial area because, the increasing trend of Pb may due to the exhausts of vehicles, industrial waste *i.e.* Dye industries, tanneries, batteries. Among all sites of experiments of bioaccumulation Mumfordganj site having the lowest concentration of As and Pb in sewage-irrigated soils because of low heavy metals content sewage-irrigated water discharge from Mumfordganj of non-industrial area. Hence, shows that long-term and indiscriminate application of industrial area discharge sewage-irrigated which contains a high concentration of As and can cause high uptake of heavy metal in surface and sub-surface soil. The present studies were performed to identify the ability of pot marigold (*Calendula officinalis* L.) for the phytoremediation of As and Pb from contaminated soils. The heavy metal bioaccumulations have highly accumulated As 74.67% and Pb 78.60% founded in *Calendula officinalis* L. crop in Naini regions, Prayagraj. The obtained results show that pot marigold (*Calendula officinalis* L.) has the ability to accumulate As and Pb both in root and shoot. The result obtained from the present investigation may be very useful for determining soil quality and it can help in remediation purposes. The study concluded that *Calendula officinalis* L. grown in sewage irrigated soils bioaccumulated As and Pb which pose a potential for phytoremediation of As and Pb in the sewage irrigated soils.

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