

Original Article

EVALUATION OF ACCUMULATED HEAVY METALS IN SOIL AND PLANT BODIES OF *Oryza sativa* L. and *Triticum vulgare* L.

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ABSTRACT

The Rice and Wheat crops are very important edible species in India, which were also stable food for diet of all the human beings. The main objective of the work is to evaluate the accumulated heavy metal levels in soil and plant bodies through pot culture experiments. The experiments were conducted by in-vitro condition in the department laboratory. The effluents samples were collected the karakambadi and renigunta industrial areas, Andhra Pradesh, India. It has been observed that the crop plant parts were affected by the effluents which were impacted on shoot, stem and root of rice and wheat crop plant parts. The statistical values of plant samples have significantly reduced when compared with control. In this study, water crop plants were subjected to higher doses of lead and cadmium, leads to significantly declined in the growth. The results suggests that water crops are not affected by oxidative stress, in spite of the presence of higher dose of lead and cadmium in the hydroponic medium, as would be anticipated for a species that has efficiently survived in a highly polluted environment.

Keywords: pot culture, Lead, Cadmium, industrial effluent.

INTRODUCTION

Heavy metal contamination of soil results from anthropogenic such as mining, agriculture as well as natural activities. Chemical and metallurgical industries are the most important sources of heavy metals in the environment. The metals are classified as "heavy metals" if in their standard state they have a specific gravity of more than 5 gr/cm³. Heavy metals get accumulated in time in soils and microbes. The heavy metal toxicity of Lead, cadmium and mercury are toxic even in very low concentrations. Every 1000 kg of "normal soil" contains 200 gr Chromium, 16 gr Pb, 0.5gr Hg and 0.2gr Cd, theoretically monitoring the endangerment of soil with heavy metals are interest due to their influence on groundwater and surface water and also on plants, animals and humans. Albeit the development of scientific and industrial technology has provided a large number of benefits to the society, it has also generated different kinds of several undesirable environmental pollutants including heavy metals. Heavy metals are being generated by different kinds of industries and reaching in the soil through industrial effluent. The quality and concentration of the heavy metals varies from the industry to industry and has a direct concern with nature of the product. Heavy metals are the stable metals or metalloids whose density is greater than 4.5 gr/cm³, namely Pb, Cu, Ni, Cd, Zn, Hg and Cr etc. They are stable and cannot be degraded or destroyed, and therefore they tend to accumulate in soils and sediments. However the soil contaminants due to their widespread occurrence, acute and chronic toxicity.

These metals are extremely persistent in the environment. They are non-biodegradable, non thermo-degradable and thus readily accumulate to toxic levels. Since they do not break down, they might affect the biosphere for a long time. It is known that heavy metals form an important polluting group. They have not only toxic and carcinogenic effect but also tend to accumulate in living organisms. However, these heavy metals were found in minimum levels in the plants grown in that specific area. Rahmani *et al.*, [1] reported that organic contents were higher in the crop irrigated with municipal wastewater while heavy metals i.e. Fe, Mn, Cd, Zn were found higher than the normal range. Mustafa *et al.*, [2] made an extensive study on impact of irrigation of sewage along the main discharge channel of Konya, Iran and found increased concentration of heavy metals such as Zn, Cu, Cr, Mn, Cr, Ni, Pb and Cd in fertile soil and showed negative effect on plant system. Mishra and Tripathi *et al.*, [3] studied heavy metals contamination of soil and their bioaccumulation in the agricultural products especially vegetables irrigated with treated waste water in Varanasi, India and

found that metals concentration of Cd-3.4, Cr-56.3, Pb-123.5, Zn-122.3 and Cu-77.8 mg/kg each in soil in the selected sites. Rattan *et al.*, [4] studied the long-term impact of irrigation with sewage effluents on heavy metal content in soil, crops and groundwater. Masto *et al.*, [5] observed change in soil quality indicators under long-term sewage irrigation in a sub-tropical environment and concluded that the long-term sewage irrigation resulted in significant build-up of DTPA extractable Zn (314%), Cu (102%), Fe (715%), Mn (197.2%), Cd (203%), Ni (135.8%) and Pb (15.2%) when compared with the metals in adjacent rain fed reference soil. Lokeshwari and Chandrappa *et al.*, [6] studied the impact of heavy metal contamination of Bellandur lake on soil and cultivated vegetation and concluded that the sewage is the main source of pollution of this water body. The irrigation with sewage contaminated water containing a variable amount of heavy metals leads to increase in the concentration of metals in the soil and vegetation. The first two forms are available to the plants while the other two are potentially available in the longer term [7]. The hazards of wastewater and solid waste used in urban and peri-urban agriculture (UPA) have been categorized into three groups: biological agents, chemical and physical hazards [8]. Soil pollution comprises the pollution of soils with materials, mostly chemicals that are out of place or are present at concentrations higher than normal which may have adverse effects on humans or other organisms.

The fulfillments of the basic need (food required) of society and to facilitate more amenities (luxury product) has caused excess generation of heavy metals which ultimately find their way on the soil. Variability of heavy metal contaminants both in their forms and quantity may be due to specific conditions. Some of the major important works made by the researcher on this approach in India can be quoted. Gupta *et al.*, [9] found that leather industries located at Jajmau, Kanpur, are the major sources of heavy metal contaminations in the agricultural soil in the surrounding areas where treated effluent has been used for irrigation. Rattan *et al.*, [4] reported that under Keshopur effluent irrigation scheme, in Delhi, India for 20 years resulted in to significant build up of DTPA-extractable Zn (208%), Cu (170%), Fe (170%), Ni (63%), and Pb (29%) in sewage irrigated soils.

PLANTS MANAGEMENT OF METAL CONTAMINATED IN SOIL

Plants have been used to stabilize or remove metals from soil and water. It has been demonstrated that plants are effective in cleaning up contaminated soil [10]. Phytoremediation is a general term for using plants to remove or degrade different kinds of soil pollutants such as

heavy metals, pesticides, solvents, crude oil, polyaromatic hydrocarbons, and landfill leachates e.g. prairie grasses can stimulate the breakdown of petroleum products. In phytoremediation, accumulation and distribution of heavy metals in plant tissue are important aspects to evaluate the role of plants in remediation of metaliferous soil.

Heavy metals include lead (Pb), cadmium (Cd), nickel (Ni), cobalt (Co), iron (Fe), zinc (Zn), chromium (Cr), iron (Fe), arsenic (As), silver (Ag) and the platinum group elements. Industrialization and urbanization have increased the anthropogenic contribution of heavy metals in the biosphere. Heavy metals have the largest availability in soil and aquatic ecosystems and to a relatively smaller proportion in the atmosphere as particulate or vapors. Heavy metal toxicity in plants varies with plant species, specific metal, concentration, chemical form and soil composition and P^H, as many heavy metals are considered to be essential for plant growth. Some of these heavy metals like Cu and Zn either serve as cofactor and activators of enzyme reactions e.g., in forming enzymes/substrate metal complex or exert a catalytic property such as a prosthetic group in metalloproteinase. These essential trace metal nutrients take part in redox reactions, electron transfer, and structural functions in nucleic acid metabolism. Some of the heavy metal such as Cd, Hg is strongly poisonous to metal-sensitive enzymes, resulting in growth inhibition and death of organisms.

MATERIALS AND METHODS

Acetone, Mercuric chloride (HgCl₂), Potassium hydroxide (KOH), Sulphuric acid (H₂SO₄), Hydrochloric acid (HCl), Hydro chloric acid (HNO₃), Whatman41 filter paper.

The plant samples of *Oryza sativa* L. and *Triticum vulgare* L. seeds were obtained from N.G. Ranga agricultural regional research station Tirupati, Andhra Pradesh, India. Seeds were surface sterilized with 0.1% HgCl₂ solution for 10 min and rinsed with double distilled water. Seeds were soaked in earth pots (30 cm x 25 cm, diameter and deep) containing red soil with pharmaceutical and battery industrial effluents. The samples were collected the Amaraja battery industry and renigunta industrial area. The pots were kept under natural photo radiation and each pot contained 20 seedlings. The pot culture of rice and wheat were analyzed, the plant parts of the shoot, stem, and root for their changes of growth and yielding of three intervals of 10th, 20th and 30th-day experimental period.

POT CULTURE EXPERIMENTS

Rice (*Oryza sativa* L.) and Wheat (*Triticum vulgaries* L.) cultivar plants were grown in pots in untreated soil (control) and in soil to which industrial effluents had been applied.

DIGESTION METHOD

Before digestion to analyze trace metals samples were dried at 70 °C for 36h. Three digestion methods were applied here involving a microwave, dry ashing and leaching. All methods were performed in triplicate for samples.

MICROWAVE ACID DIGESTION

Preparation of Soil Samples

The soil samples (30 cm depth) were collected adjacent to the plant roots. The pH was determined in aqueous extract 1:2. Organic matter was determined by a colorimetric method using K₂Cr₂O₇, H₂SO₄, and saccharose solution as a standard solution. Total P was determined by elemental analysis. Electrical conductivity was measured by a digital conductivity meter (Elico CM 180), with a cell constant value of 1.0. For the determination of trace metals, soil samples were dried at 400 °C to constant weight, sieved to a particle size of 2 mm, subjected to microwave acid digestion (Milestone ETHOS ONE) with HNO₃: HCL: H₂O₂:HF (4:1:3:2) and evaporate to dryness to remove Hydrogen fluoride.

To 0.1 gram of soil sample 3 mL of nitric acid, 2 mL of hydrochloric acid and 1 mL of hydrogen fluoride was added for dissolution [11]. And later subjected to microwave acid digestion (Milestone Ethos One).

Preparation of Plant Sample

The plant species named *Oryza sativa* L. and *Triticum vulgare* L. were collected .The collected plants were washed with running tap water in

order to remove the adhering soil particles and then separated the plant bodies and dried at 60±500 °C until constant weight and then ground and sieved. Later samples were kept for ashing in a muffle furnace (PYRO, Milestone) to remove the carbon content then digested with HNO₃: HCL: H₂O₂ (10:2:5). To 1 gram of plant sample 5 mL of nitric acid and 2 mL of hydrogen peroxide were added [11].

RESULTS

The levels of heavy metal in industrial effluents like pharmaceutical and battery industries, which were shown in the Table-1 and Fig-1. The levels if heavy metals like Ni, Fe, Zn, Cd, Pb, Cr levels in pharmaceutical industries were 96.3, 0.00, 0.00, 23.6, 0.060, 0.619 and battery industries 89.6, 120.3, 0.00, 46.3, 0.182, 0.822 level concentrations were observed (Table-1 and Fig-1)

Table 1: Heavy metal levels in industrial effluents (mg / l).

S No	Metals	Pharmaceutical Industrial Effluents	Battery Industrial Effluents
1	Ni	96.3	89.6
2	Fe	0.00	120.3
3	Zn	0.00	0.00
4	Cd	23.6	46.3
5	Pd	0.060	0.182
6	Cr	0.619	0.822

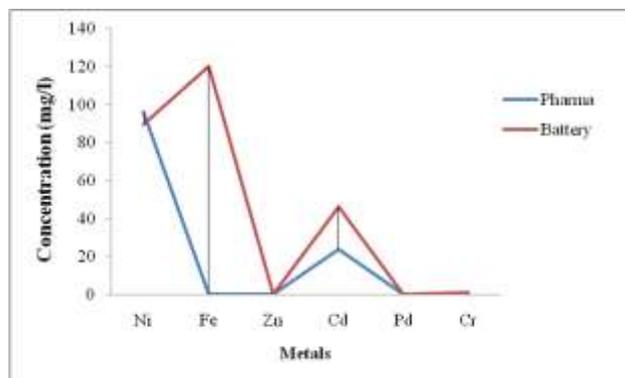


Fig.1: Heavy metal concentration in industrial effluents.

The levels of heavy metal in red soil, which were shown in the Table-2 and Fig-2. The levels if heavy metals like Mn, Fe, Cu, Zn, Ni, Pb, Cd levels in red soil were 11.51, 4.21, 0.118, 0.344, 0.302, 0.166, 0.036 concentrations were observed (Table-2 and Fig-2).

Table 2: Metals contamination of (mg /l) in the red soil.

S No	Metals	Concentrations (mg/l)
1	Mn	11.51
2	Fe	4.21
3	Cu	0.118
4	Zn	0.344
5	Ni	0.302
6	Pb	0.166
7	Cd	0.036

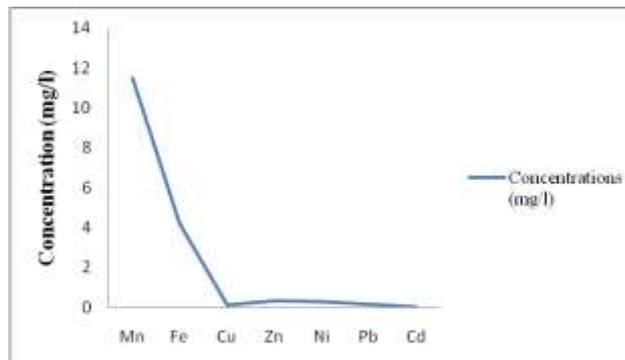


Fig.2: Heavy Metals levels in Red soil

Incorporation of Cadmium contents in Rice

Statistically extreme significant changes in Cadmium levels were observed in the Rice of industrial effluents-treated different areas of plant parts. In the Control groups, the Cadmium levels were 0.093, 0.097, and 0.098 observed in the shoot, stem and roots of rice. And increased deposited Cadmium levels in Pharmaceutical effluents-treated group Cadmium levels were 0.095, 0.099, 0.113 and 2.20% higher than the control and 2.27, 13.27% increased than in the shoot treated Pharmaceutical industrial effluents treated. And increased deposited Cadmium levels in Battery industrial effluents-treated group Cadmium levels, were 0.105, 0.125, 0.151 and 11.42% higher than the control and 22.5, 35.04% higher cadmium levels were observed in the stem, root and which were lesser than in the shoot treated Battery industrial effluents treated (Table-2 and Fig-3).

Incorporation of Cadmium contents in Wheat

Statistically extreme significant changes in Cadmium levels were observed in the Wheat of industrial effluents-treated different areas of plant parts. In the Control groups, the Cadmium levels were 0.115, 0.123, and 0.132 observed in the shoot, stem and roots of Wheat. And increased deposited Cadmium levels in Pharmaceutical effluents-treated group Cadmium levels were 0.124, 0.131, 0.147 and 7.25% higher than the control and 6.10, 10.20% increased than in the shoot treated Pharmaceutical industrial effluents treated. And increased deposited Cadmium levels in Battery industrial effluents-treated group Cadmium levels, were 0.158, 0.178, 0.213 and 27.21% higher than the control and 30.89, 38.02% higher cadmium levels were observed in the stem, root and which were lesser than in the shoot treated Battery industrial effluents treated (Table-3 and Fig-3).

Table 3: Incorporation of Cadmium contents in Rice and Wheat crop plant parts

S.No.	Rice			Wheat		
	Shoot	Stem	Root	Shoot	Stem	Root
Control	0.093	0.097	0.098	0.115	0.123	0.132
	±	±	±	±	±	±
Pharmaceutical industrial effluent	0.004	0.006	0.005	0.005	0.005	0.006
	0.095	0.099	0.113	0.124	0.131	0.147
Battery industrial effluent	±	±	±	±	±	±
	0.004	0.003	0.004	0.005	0.004	0.004
	(-2.10%)	(-2.02%)	(-13.27%)	(7.25%)	(-6.10%)	(-10.20%)
Battery industrial effluent	0.105	0.125	0.151	0.158	0.178	0.213
	±	±	±	±	±	±
	0.005	0.006	0.006	0.006	0.028	0.004
	(-11.42%)	(-22.4%)	(-35.09%)	(-27.21%)	(-30.89%)	(-38.02%)

Average of Six replications; Per cent over control values are given in parentheses

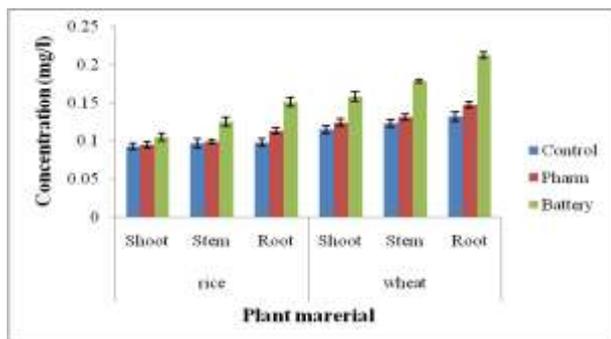


Fig.3: Cadmium levels in *Oryza sativa* L. and *Triticum vulgare* L. crop plant parts. (Data are expressed as mean± SD).

Incorporation of Lead contents in Rice

Statistically extreme significant changes in Lead levels were observed in the Rice of industrial effluents-treated different areas of plant parts. In the Control groups, the Lead levels were 0.272, 0.316, and 0.332 observed in the shoot, stem and roots of rice.

And increased deposited Lead levels in Pharmaceutical effluents-treated group Lead levels were 0.326, 0.341, 0.360 and 16.56% higher than the control and 7.33, 3.33% increased than in the shoot treated Pharmaceutical industrial effluents treated. The increased deposited Lead levels in Battery industrial effluents-treated group Lead levels, were 0.354, 0.421, 0.463 and 23.16% higher than the control and 24.94, 28.29% higher Lead levels were observed in the stem, root and which were lesser than in the shoot treated Battery industrial effluents treated (Table-4 and Fig-4).

Incorporation of Lead contents in Wheat

Statistically extreme significant changes in Lead levels were observed in the Wheat of industrial effluents-treated different areas of plant parts. In the Control groups, the Lead levels were 0.367, 0.383, and 0.397 observed in shoot, stem and roots of Wheat, and increased deposited Lead levels in Pharmaceutical effluents-treated group Lead levels were 0.393, 0.458, 0.497 and 6.61% higher than the control and 16.37, 20.12% increased than in the shoot treated Pharmaceutical industrial effluents treated, and increased deposited Lead levels in Battery industrial effluents-treated group Lead levels, were 0.501, 0.572, 0.601 and 26.74% higher than the control and 33.04, 33.94% higher Lead levels were observed in stem, root and which were lesser than in the shoot treated Battery industrial effluents treated (Table-4 and Fig-4).

Table 4: Incorporation of Lead in *Oryza sativa* L. and *Triticum vulgare* L. crop plant parts

S.No.	<i>Oryza sativa</i> L.			<i>Triticum vulgare</i> L.		
	Shoot	Stem	Root	Shoot	Stem	Root
Control	0.272	0.316	0.332	0.367	0.383	0.397
	±	±	±	±	±	±
Pharma industrial effluent	0.049	0.003	0.004	0.004	0.003	0.004
	0.326	0.341	0.360	0.393	0.458	0.497
Battery industrial effluent	±	±	±	±	±	±
	0.003	0.004	0.003	0.008	0.003	0.005
	(-16.56%)	(-7.33%)	(-7.77%)	(-6.61%)	(-16.37%)	(-20.12%)
Battery industrial effluent	0.354	0.421	0.463	0.501	0.572	0.601
	±	±	±	±	±	±
	0.048	0.003	0.004	0.004	0.002	0.003
	(-23.16%)	(-24.94%)	(-28.29%)	(-26.74%)	(-33.04%)	(-33.94%)

Average of Six replications: Per cent over control values are given in parentheses.

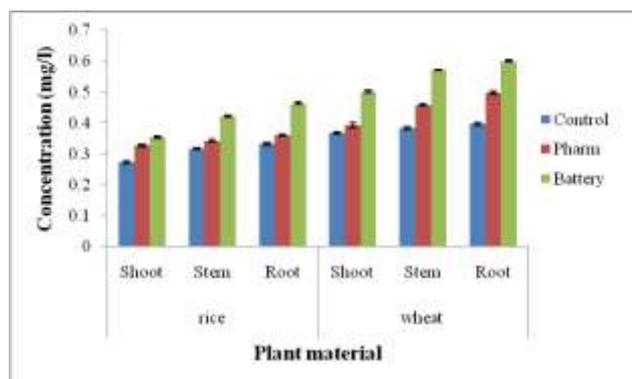


Fig.4: Lead contents in *Oryza sativa L* and *Triticum vulgare L.* crop plant parts. (Data are expressed as mean \pm SD).

DISCUSSION

The exposure of plants to toxic levels of heavy metals triggers a wide range of physiological and metabolic alterations [12, 13]. However, as different heavy metals have different sites of action within the plant, the overall visual toxic response differs between heavy metals. The most widespread visual evidence of heavy metal toxicity is a reduction in plant growth [14] including leaf chlorosis, necrosis, turgor loss, a decrease in the rate of seed germination, and a crippled photosynthetic apparatus, often correlated with progressing senescence processes or with plant death. All these effects are related to ultrastructural, biochemical, and molecular changes in plant tissues and cells brought about by the presence of heavy metals [15]. Contamination of agricultural soil by heavy metals has become a critical environmental concern due to their potential adverse ecological effects. Such toxic elements are considered as soil pollutants due to their widespread occurrence and their acute and chronic toxic effect on plants grown of such soils.

The regulatory limit of cadmium (Cd) in agricultural soil is 100 mg/kg soil [16]. Plants grown in soil containing high levels of Cd show visible symptoms of injury reflected in terms of chlorosis, growth inhibition, browning of root tips and finally death [17]. The inhibition of root Fe (III) reductase induced by Cd led to Fe (II) deficiency, and it seriously affected photosynthesis. In general, Cd has been shown to interfere with the uptake, transport and use of several elements (Ca, Mg, P and K) and water by plants. Cd also reduced the absorption of nitrate and its transport from roots to shoots, by inhibiting the nitrate reductase activity in the shoots.

In this study the result shows that the germination of rice and wheat seeds were inhibited considerably with increasing heavy metal concentrations. Therefore, under field conditions, soil Cd pollution may have adverse effects on seed germination. In higher plants, Cd toxicity generally inhibits the growth and reduces the biomass production. The germination did not decrease in response to heavy metals exposure. Heavy metals stress caused a significant inhibition of root elongation and seedling biomass. Less toxicity has been seen in shoot. Blum *et al.*, [18] also found root length to be the most sensitive parameter to Cd and Pb treatment. Inhibition of root elongation is considered to be the first evident effect of Cd and Pb toxicity in plants due to the fact that plant roots are the first point of contact with the toxic Cd and Pb in the growth medium [19]. Cell divisions at the root tip and cell elongation in the extension zone were affected by the presence of heavy metals. Cd-induced inhibition of root growth was associated with a decrease of K, Mg, Ca, and Fe concentrations in roots [19]. Cd was found accumulated in the root and leaf. The accumulation of Cd in the root and leaf depends on binding to the extracellular matrix and on the transport efficiency. Results of this research showed that the accumulation of Cd and Pb in roots were higher than in leaves (Fig-3 and 4). Most of the Cd and Pb that entered the plant system accumulated in the roots. A first barrier against Cd and Pb stress, operating mainly at the root level, can be immobilization of Cd and Pb by means of the cell wall and extracellular carbohydrates.

Minimum concentrations of heavy metals were found in stem as it is a transporter organ. Plants may accumulate and store metals in root and stem in non-toxic forms. Binding of toxic metals at cell walls of roots

and leaves away from sensitive sites within the cell or storing them in a vacuolar compartment are known avoidance mechanisms of heavy metal tolerance in plants [20]. It is well known that mobilization of seed reserves, which occurs during early seed germination, is crucial because it supplies substrates essential to growth of the root and plumule.

CONCLUSION

In conclusion, an efficient adaptation to industrial effluents and the valuable Lead, Cadmium accumulation observed for water crop plants, especially at higher doses of heavy metal, shows the great potential of this plant species for the decontamination of pollutants in water-based systems. In this study, water crop plants adapted to higher doses of Lead, Cadmium deposition leads to significantly decreases growth was observed. The results suggested that water crops are not affected by oxidative stress, in spite of the presence of higher dose of Lead, Cadmium in the hydroponic medium, as would be anticipated for a species that has efficiently survived in a highly polluted environment.

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