P: ISSN No. 2231-0045 RNI No. UPBIL/2012/55438 VOL.-7, ISSUE-2, November-2018

E: ISSN No. 2349-9435 Periodic Research

Phytoremediation by Paddy Crop of Heavy Metals (Cr, Pb &Ni) In Tannery Waste and Sewage Sludge-Amended Soil

Radhey Shyam
Designation,
Deptt. of Chemistry,
D.B.S. (P.G.) College,
Dehradun, India

Neeraj Kumar
Designation,
Deptt. of Chemistry,
S.S.M.V. (P.G.) College,
Shikarpur, Bulandshahr, India

Abstract

Several approaches are currently being used for the prevention, control and remediation of soil contaminated with toxic metals. These include, (a)land filling - excavation, transport and deposition of contaminated soil in a permitted land fill site (b) chemical immobilization of heavy metals by the application of ameliorants like lime, farmyard manure (FYM), phosphates, manganese oxides *etc.,* (c) leaching - using acid solutions or complexing leachants (EDTA *etc.)* to desorb and leach metals from the soil drawn from the contaminated area followed by the return of the soil residues to the site; (d) bioremediation - use of micro-organisms to degrade pollutants in site (since the heavy metals cannot be chemically degraded, application of microbial remediation to the in-site removal of heavy metals from the contaminated substrates is limited mainly to their immobilization by precipitation or reduction); and (e) phyto-remediation- use of specially selected and engineered metal-accumulating plants for environment clean-up either by phytoextraction or by phytostabilization. Among these, soil excavation is the only method for the total removal of heavy metals from contaminated soil. However, it can not be recommended as a viable practice, since it is prohibitively expensive. Thus, chemical immobilization of heavy metals by the application of ameliorants (lime, phosphates, FYM *etc*.), use of complexing extractant/leachants (e.g. EDTA.) and phyto-remediation appear to be the potential remedial measures to alleviate the heavy metal level in soil. Metal concentration in crops is mostly not high enough to cause acute toxicity, but in the long run it may provoke chronic damage to health. Due to the heavy metal burden in human nutrition, there is a need for measures to reduce the metal transfer into agricultural plants. In areas where conventional or other remediation technologies are eithernot feasible or too expensive, other simple but effective approaches may help to reduce the accumulation of heavy metals in the edible parts of crops.

Keywords: Phytoremediation, Complexing Agent and Heaving Meta.

Introduction

Phytoremediation is the use of crop plants to absorb and remove metal contaminants from the soil. Some crop plants tend to concentrate a specific metal of the heavy metals and allow its removal and safe disposal at the time of harvest. The metalextractability and accumulating ability of crop species is also influenced by the addition or presence of soil amendments and complexing agents. The aspects of heavy metal accumulation by crop species particularly in tannery waste and sludge-amended soil under different soil moisture conditions in the presence of lime and complexing agents have not been extensively studied. Chemically enhanced phytoextraction has been developed to overcome these problems (Huang and Cunningham, 1996; Blaylock at *al.,* 1997; Huang *et al.*, 1997; Blaylock, 2000). This approach makes use of high biomass crops that are induced to take up large amounts of metals when their mobility in soil is enhanced by chemical treatments. Several chelating agents, such as citric acid, EDTA, CDTA, DTPA, EGTA, EDDHA and NTA, have been studied for their ability to mobilize metals and increase metal accumulation in different plant species (Huang *et al*., 1997; Cooper *et al*., 1999). Different metals such as Pb (Blaylock *et al*., 1997; Huang *et al*., 1997), U

(Huang *et al*., 1998), Cs^{137} (Lasat *et al.*, 1998), and Au (Anderson *et al*., 1998) have been targeted, the most promising application of this technology at the moment is for the remediation of Pb contaminated soils using Indian mustard [*Brasicca juncea(L.) Czern*] in combination with EDTA (*e.g.* Blaylock, 2000). Despite the success of this technology, some concern has been expressed regarding the enhanced mobility of metals in soil and their potential risk of leaching to ground water (Cooper *et al.*, 1999). However, no detailed studies regarding the persistance of metal-EDTA complexes in contaminated soils have been conducted.

Objectives

To assess the effectiveness of lime and complexing agent (EDTA) and to compare the plant species rice in relation to the availability and accumulation of heavy metal in plants in tannery waste and sewage sludge – amended soil.

Review of Literature

It is one of the toxic metals in nature and affected the plant metabolism adversely during the use of Pb containing irrigation water or Pb contaminated soils. Wyszkowska et al. (2007) reported that in 50mg/kg Pb contaminated soil reduced the oat crop yield 60% in heavy loamy sand soil. This reduction is less in light silty loam soil compared to loamy sand. The increasing level of Pb concentration upto a level plant could assimilate significant amount of Pb in body tissues from soil to plant, but higher doses of Pb reduced the soil microbial biomass and harm the plant root which showed decline rate of Pb uptake. Liu et al. (2004) reported that a reduction in the formation of new cells under the influence of Pb leads to a reduction in shoot and root lengths. Similar type of result was also reported by many researchers in spinach (Khan et al., 2008a, b; Dhongade et al., 2011) and rice crop (Yizong et al., 2009). Kalaivanan and Ganeshamurthy (2016) described that impact of heavy metals on microorganisms and on enzymatic activity depends, among others, on soil pH, content of organic and mineral colloids, as well as on the type of heavy metals and their chemical properties. Kucharski and Wyszkowska (2004) stated that the heavy metal uptake was affected by the soil factors as well as plant parts. Leafy vegetables accumulated maximum levels of Pb, followed by root vegetables. Wyszkowska et al. (2007) described that soil contamination with heavy metals (Cr, Pb, Hg) reduced the population size of Azotobacter spp. The counts of other microbial groups, i.e. copiotrophic bacteria, spore-forming copiotrophic bacteria, oligotrophic bacteria, spore-forming oligotrophic bacteria, ammonifying bacteria, nitrogen immobilizing bacteria, cellulose-decomposing bacteria, Arthrobacterspp., Pseudomonasspp., actinomyces and fungi, showed varied susceptibility to heavy metals. The interaction effect of different metals also affects the growth and uptake pattern of other plant nutrient in plants. Pandey and Sharma (2002) provided abundant information on the influence of single heavy metals on soil metabolism as well as on the growth and development of different plant species. However, data on the combined effects of several heavy metals on the microbiological and biochemical properties of soil as well as on plants are scarce (Giridhara and Siddaramappa, 2002); still in a natural environment heavy metal pollution is in most cases caused by some heavy metals. In the present context, use of nanomaterial for the removal of Pb from water bodies is a major area of research at the top of the global issues. Use of titanium oxide and hematite nanoparticles is the foremost, for the 100% recovery of the Pb ions. This efficiency also affected by the pH and contact time; which is ≤ 6 and ≥60 min, respectively, for the typical optimum conditions for Pb removal of water bodies. The recovery per cent also affected by adsorbent dose for the adequate surface area and number of adsorption sites (Bhatia et al., 2016). The interactive effect of various metals is also negatively affected the growth of the plants; 50mg /kg Pb contaminated soil reduced the oat crop yield 60% when the Ni level applied 200 mg/kg in heavy loamy sand soil (Wyszkowska et al., 2007).

Materials and Methods

The pot culture experiments involved growing of paddy in sewage sludge and tannery waste-treated soil with the addition of amendments ($CaCO_3$ and EDTA).

Treatment details

The following treatments were paddy under submerged soil moisture regime

Organic Wastes**:** Two

(a) Sewage Sludge

(b) Tannery Waste

Levels of organic waste: Two

(a) 1.5% : 60g organic waste per pot

(b) 3.0 % : 120 g organic waste per pot

Amendments: Three

(a)No amendment (organic waste alone)

(b) Calcium carbonate (5%):200 g $CaCO_3$ per pot

(a) Ethylene diamine tetra acetic acid (0.1%): 4 g EDTA per pot

Replications: Three

Experimental design

Completely Randomized Design (Factorial)

Treatments combination

T_1: Control
T_2: Sewage Sludge (1.5%)
T_3: Sewage Sludge (1.5%) + $CaCO_3$
T_4: Sewage Sludge (1.5%) + EDTA
T_5: Sewage Sludge (3.0%)
T_6: Sewage Sludge (3.0%) + $CaCO_3$
T_7: Sewage Sludge (3.0%) + EDTA
T_8: Tannery Waste (1.5%)
T_9: Tannery Waste (1.5%) + $CaCO_3$
T_{10}: Tannery Waste (1.5%) + EDTA
T_{11}: Tannery Waste (3.0%)
T_{12}: Tannery Waste (3.0%) + $CaCO_3$
T_{13}: Tannery Waste (3.0%) + EDTA

Pot Culture Experiment (*Karif*)

Pot culture experiment with paddy was conducted in set of 5 kg capacity, pots in the Net house set of 39 pots was maintained for paddy.

Filling of Pots with Soil and Addition of Treatments

Four kg of processed soil was filled in each of the 39 plastic pots (capacity, 5 kg) and required amount of sewage sludge and tannery waste according to the treatments was added and mixed in pots. Water was added to each pot and kept standing 1 cm. above the soil surface to have the submerged soil moisture condition upto 12 months period and pots soil contents were mixed thoroughly with wooden stick. The whole set-up of pots was left as such for 12 months duration with intermittent disturbance of soil contents with wooden stick. Later, the required amounts of $CaCO_3$ and EDTA as per the treatment detailed below was added to pots and mixed thoroughly with wooden stick.

Preparation of Paddy Nursery

A starter dose of nutrients (N-30 kg, P_2O_5- 25 kg and K_2O- 30 kg ha^{-1}) and adequate amount of farm yard manure was added to the required amount of soil, mixed thoroughly and filled in two cemented beds (1m x 0.5m). Bold and healthy paddy seed (var. P-10) in sufficient quantity were sown in beds at suitable moisture content. Irrigation was given and weeding was performed as and when required during the nursery preparation.

Transplanting of Paddy Saplings

Half dose of N 75 kg N ha^{-1}) and full dose of P_2O_5 (60 kg ha^{-1}) and K_2O (50 kg ha^{-1}) were applied to each pot in the form of urea, DAP and MOP solution. Paddy (var. P-10) obtained from nursery were washed carefully under tap water and transplanted in each pot. Five bunches each of 2 seedlings were planted per pot. Remaining half dose of N (75 kg ha^{-1}) was applied at 25 days after transplanting.

Harvesting of Paddy Crop

Crop plants in the pots were allowed to grow upto full flowering stage. Irrigation was given regularly in order to maintain the lowland rice conditions. Proper care against weeds, pests and diseases was taken during the entire crop growth period. The crop was harvested at flowering stage, first dried in air and then in hot air oven at 70 ^{0}C till constant weight. The dry matter weight of plant material per pot was recorded.

Plant Analysis

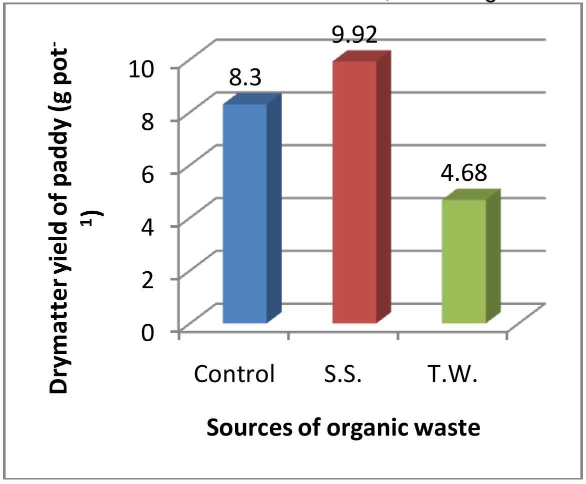
The oven-dried plant samples were ground with the help of a stainless steel grinder for subsequent analysis. Two- gram quantity of ground plant material was taken in 100 ml conical flasks, first predigested with HNO_3 and later digested with diacid mixture of HNO_3:$HClO_4$ (5:1) on an electric hot plate. Digested material was cooled, diluted with double distilled water and filtered through Whatman No.1 filter paper in to 100 ml volumetric flask and then the volume was made upto the mark with double distilled water. The plant digests thus obtained were analyzed for Pb, Ni and Cr using Inductively Coupled Argon Plasma-Atomic Emission Spectrophotometer (ICAP-AES) at the Department of Soils, Punjab Agricultural University, Ludhiana, (Punjab).

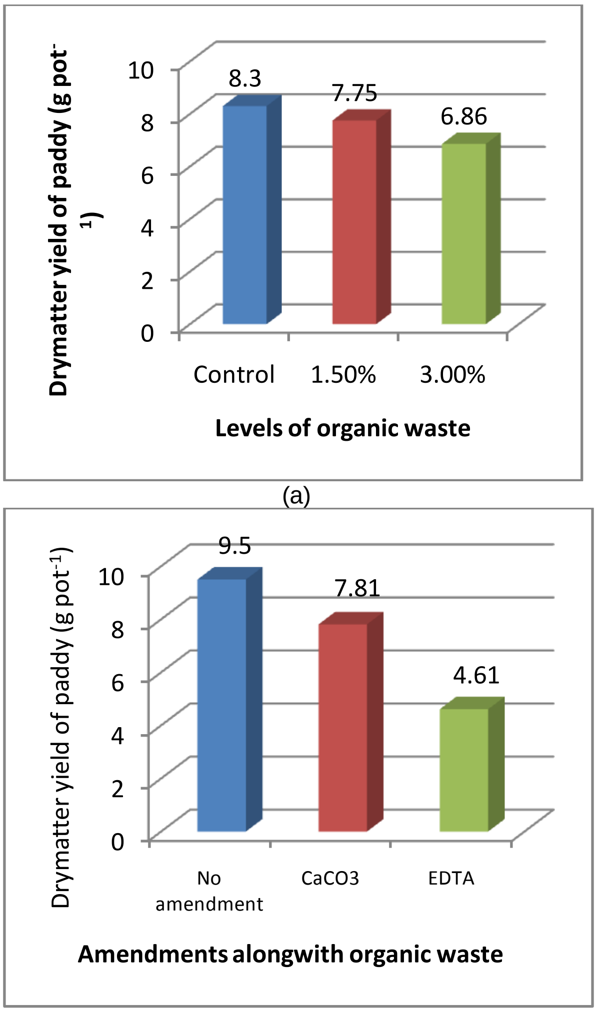
Result

Data in table 1 clearly indicate that sewage sludge at 1.5% (T_2) or 3.0 % level (T_5) applied either alone or in combination with $CaCO_3$ (T_3 and T_6) had significantly higher dry matter yield over control (T_1). The dry matter yield with these treatments ranged from 10.13 g pot^{-1} (T_6) to 13.8 g pot^{-1} (T_5) as against 8.3 g pot^{-1} obtained with control (T_1). The extent of increase in biomass yield over control varied from 21.04 to 60.24 %. All the treatments of tannery waste irrespective of its levels applied in combination with $CaCO_3$ and EDTA (T_8, T_{11}, T_9, T_{10}, T_{12}, and T_{13}) and also treatments of sewage sludge applied along with EDTA (T_4 and T_7) had significantly lower drymatter yield as compared to control. The variation in drymatter yield among these treatments ranged from 2.5 g pot^{-1} (T_{13}) to 6.9 g pot^{-1} (T_8) plate1.

Plate 1: A general view of the pot culture experiment with paddy

Data on the influence of different factors *viz.* organic wastes, their levels, amendments and their interactions on drymatter yield of paddy at flowering have been demonstrated in table 2a, b and Fig. 1.





(a)

(b)

Fig.1: (a) Effect of sources and levels of organic waste and (b) amendments on mean drymatter yield of paddy at flowering

Addition of sewage sludge resulted in higher drymatter yield of paddy at flowering (9.92 g pot^{-1}) and proved significantly superior to tannery waste (4.68g pot^{-1}). Between the two level of organic wastes, 1.5% level produced significantly greater drymatter yield (7.75 g pot^{-1}) in comparison to 3.0% level (6.86 g pot^{-1}). The interaction between levels and wastes was significant (Table2a). Application of 3.0% level of sewage sludge (9.82 g pot^{-1}) significantly enhanced the biomass production of paddy at flowering as compared to the addition of 1.5% level of tannery waste (5.47 g pot^{-1}).

It is evident from the data shown in Table 2b that addition of amendments (CaCO$_3$ and EDTA) along with organic waste significantly reduced the drymatter yield of paddy (7.81 and 4.61 g pot^{-1} respectively) as compared to the drymatter yield obtained with plain application of organic waste (9.5 g pot^{-1}). However, between the two amendments the magnitude of reduction in yield was significantly more with EDTA (4.6 g pot^{-1}) than with CaCO$_3$ (7.81 g pot^{-1}). The interaction between wastes and amendments was significant with respect to drymatter production of paddy at flowering.

Table 1. Effect of various treatments of organic wastes on drymatter yield of paddy at flowering

Code no.	Treatments	Drymatter yield (g pot^{-1})
T$_1$	Control	8.30
T$_2$	S.S.(1.5%)	12.30
T$_3$	S.S.(1.5%) + CaCO$_3$ ((5%)	11.10
T$_4$	S.S. (1.5%) + EDTA (0.1%)	6.70
T$_5$	S.S. (3%)	13.80
T$_6$	S.S. (3%) + CaCO$_3$ (5%)	10.13
T$_7$	S.S. (3%) + EDTA (0.1%)	5.53
T$_8$	T.W. (1.5%)	6.90
T$_9$	T.W. (1.5%) + CaCO$_3$ (5%)	5.80
T$_{10}$	T.W. (1.5%) + EDTA (0.1%)	3.70
T$_{11}$	T.W. (3%)	5.00
T$_{12}$	T.W. (3%) + CaCO$_3$ (5%)	4.20
T$_{13}$	T.W. (3%) + EDTA (0.1%)	2.50

S.Em.(±) 0.52
C.D. (5%) 1.54

Table 2 a. Interaction Effect of Levels and Sources of Organic Waste on The Drymatter Yield (G Pot^{-1}) Of Paddy At Flowering

Sources of organic waste	Levels of organic waste		Mean
	1.5%	3.0%	
Sewage sludge	10.03	9.82	9.92
Tannery waste	5.47	3.90	4.64
Mean	7.75	6.86	

Control: 8.30 (g pot^{-1})

	SOW	L	SOW ×L
S.Em.(±)	0.211	0.211	0.299
C.D. (5%)	0.614	0.614	0.868

Table 2b. Interaction Effect of Sources of Organic Wastes and Amendments on The Drymatter Yield (G Pot^{-1}) of Paddy at Flowering

Sources of organic waste	No amendment	Amendments		Mean
		$CaCO_3$	EDTA	
Sewage sludge	13.05	10.62	6.12	9.93
Tannery waste	5.95	5.00	3.10	4.68
Mean	9.50	7.81	4.61	

	SOW	A	SOW ×A
S.Em.(±)	0.211	0.259	0.366
C.D. (5%)	0.614	0.752	1.063

Chromium Concentration in Paddy

Data in table 3 clearly indicate that Cr concentration in plants increased significantly with the application of two sources of organic waste. The chromium contents in plants ranged from 0.55 µg g^{-1} in T_3 to 0.77 µg g^{-1} in T_5 treatments with sewage sludge and from 0.52 µg g^{-1} in T_8 to 1.20 µg g^{-1} in T_{13} treatments with tannery waste as against 0.50 µg g^{-1} with control (T_1). Sewage sludge applied alone at 1.5% and 3.0% levels (T_2 and T_5) had significantly higher Cr concentration in plants than the tannery waste applied alone at equivalent levels (T_8 and T_{11}). However, when two levels of sewage sludge applied in combination with $CaCO_3$ and EDTA (T_3,T_4,T_6 and T_7) showed significantly lower Cr content in plants in comparison to the corresponding treatments of levels and amendments applied in combination with tannery waste (T_9,T_{10},T_{12}, and T_{13}).

Data on Cr concentration in paddy plants as influenced by individual factors namely organic wastes, their levels, amendments and their interaction have been given in interaction table 4a and 4 b.

It is evident from the data shown in interaction table 4a. that addition of tannery waste (0.86 µg g^{-1}) resulted in significantly higher Cr concentration in paddy than with the incorporation of sewage sludge (0.65 µg g^{-1}). Between the two levels of organic wastes, 3.0% level (0.80 µg g^{-1}) significantly increased Cr concentration in paddy as compared to 1.5% level (0.71 µg g^{-1}). The interaction between levels and organic wastes was significant with respect to Cr content in plants (Table 4a). It was further noticed that application of tannery waste at 1.5% level (0.82 µg g^{-1}) was equivalent or better in effect to the application of sewage sludge at 3.0% level (0.69 µg g^{-1}) in relation to Cr concentration in plants.

The effect of two amendments ($CaCO_3$ and EDTA) studied along with organic wastes did not show any significant difference with respect to Cr concentration in plants. The interaction between wastes and amendments was significant (Table 4b).

Table3. Effect of Various Treatments of Organic Wastes on Chromium Concentration in Paddy at Flowering

Code no.	Cr concentration in paddy (µg g^{-1})
T_1	0.50
T_2	0.39
T_3	0.55
T_4	0.57
T_5	0.77
T_6	0.63
T_7	0.67
T_8	0.52
T_9	0.84
T_{10}	1.10
T_{11}	0.60
T_{12}	0.92
T_{13}	1.20

S.Em.(±) 0.04
C.D. (5%) 0.13

Table 4a Interaction Effect of Levels And Sources of Organic Waste on Chromium Concentration (µg G^{-1}) In Paddy At Flowering

Sources of Organic Waste	Levels of organic waste		Mean
	1.5%	3.0%	
Sewage sludge	0.603	0.689	0.646
Tannery waste	0.819	0.907	0.863
Mean	0.711	0.798	

Control: 0.50 (µg g^{-1})

	SOW	L	SOW x L
S.Em.(±)	0.018	0.018	0.026
C.D. (5.0%)	0.054	0.054	0.076

Table 4 Interaction Effect of Sources of Organic Waste and Amendments on Chromium Concentration (µg G^{-1}) In Paddy At Flowering

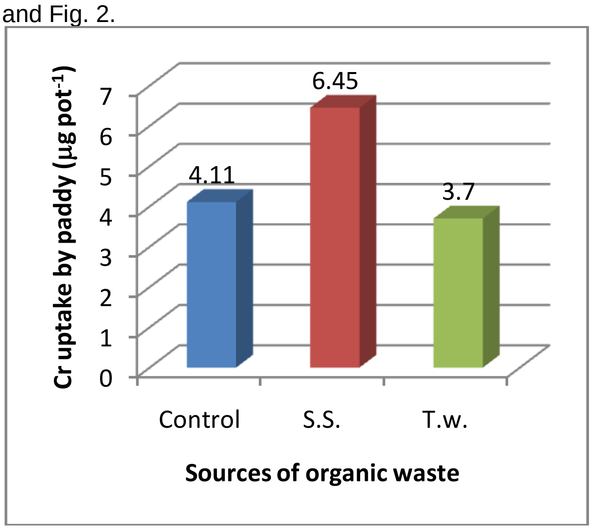
Sources of Organic Waste	No amendment	Amendments		Mean
		$CaCO_3$	EDTA	
Sewage sludge	0.728	0.592	0.618	0.646
Tannery waste	0.560	0.878	1.150	0.863
Mean	0.644	0.735	0.884	

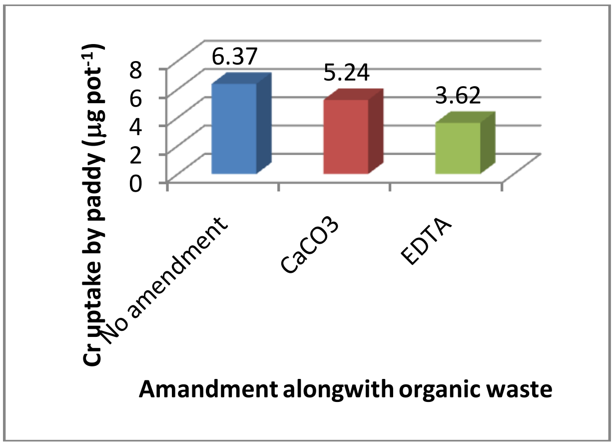
	SOW	A	SOW x A
S.Em.(±)	0.018	0.025	0.032
C.D. (5%)	0.054	N.S.	0.093

Chromium Uptake in Paddy

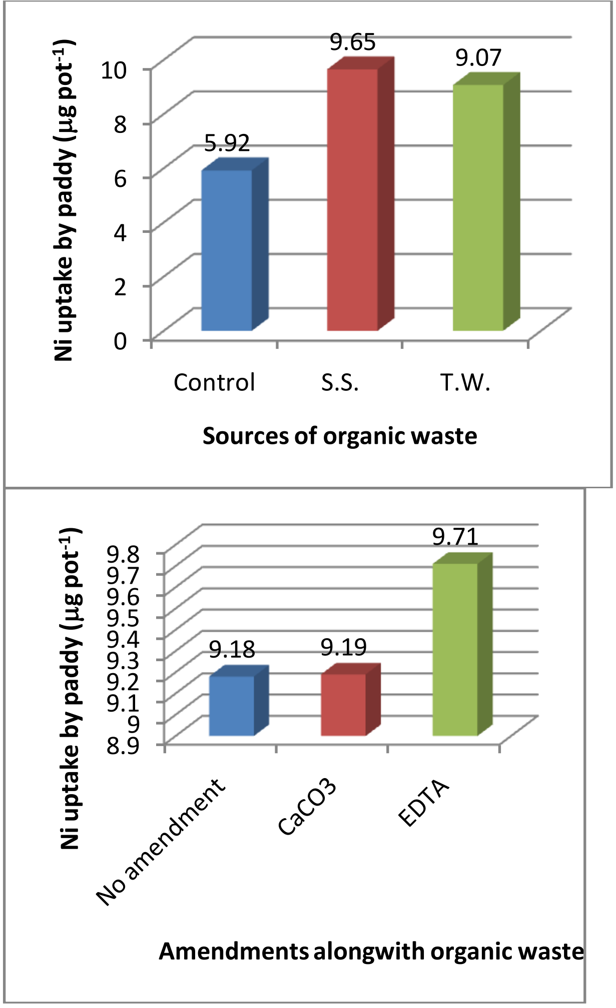
A perusal of data displayed in table 5 indicated that Cr uptake in paddy at flowering recorded with various treatments based on sewage sludge except the treatments T_4 was significantly higher than that of obtained with corresponding treatments based on tannery waste. The treatments of sewage sludge showed a variation in the values of chromium uptake ranging from 3.66 µg pot^{-1} with T_7 to 10.51 µg pot^{-1} with T_5, while a range varying from 2.96 µg pot^{-1} with T_{13} to 4.82 µg pot^{-1} with T_9 was noticed across the treatments based on tannery waste, as against 4.11 µg pot^{-1} observed with control (T_1). EDTA used an amendment in combination either with sewage sludge or with tannery waste (T_4, T_7, T_{10} and T_{13}) had Cr uptake values comparable to or significantly lower than the control (T_1).

Data on Cr uptake by paddy at flowering as affected by various factors such as sources of organic waste, their levels, amendments and their interactions have been demonstrated in interactions table 6a,b and Fig. 2.





(a)



(b)

Fig 2: Effect of Sources Of Organic Waste And Amendments On (A) Mean Cr Uptake And (B) Mean Ni Uptake By Paddy At Flowering

It is evidently clear from the observation shown in interaction table 6a that between the two organic wastes studied, the application of sewage sludge (6.45 µg pot^{-1}) resulted in significantly higher Cr uptake in paddy at flowering in comparison to tannery waste (3.70 µg pot^{-1}). The effect of levels of organic waste on Cr uptake in paddy was not significant. The interaction between the levels and sources of organic waste was significant in relation to Cr uptake in paddy.

Among the treatments of amendments, no amendment (6.37 µg pot^{-1}) caused highest Cr uptake in paddy followed by $CaCO_3$ (5.24 µg pot^{-1}) and EDTA (3.62 µg pot^{-1}) as it as evident from the interaction table 6b. The interaction between the sources of organic waste and amendment was significant in respect of Cr uptake in paddy. The application of tannery waste alone (with no amendment) (3.73 µg pot^{-1}) was as good as the application of sewage sludge in combination with EDTA (3.26 µg pot^{-1}) in relation to Cr uptake in paddy. The effect of sources of organic waste in this respect has previously been explained in table 6a.

Table 5 Effect of Various Treatments of Organic Wastes on Chromium Uptake in Paddy at Flowering

Code no.	Cr uptake in paddy (µg pot^{-1})
T_1	4.11
T_2	8.45
T_3	5.93
T_4	3.80
T_5	10.51
T_6	6.37
T_7	3.66
T_8	3.56
T_9	4.82
T_{10}	4.05
T_{11}	2.97
T_{12}	3.83
T_{13}	2.96

S.Em.(±) 0.14
C.D. (5%) 0.40

Table 6a Interaction Effect of Levels and Sources of Organic Waste on Chromium Uptake (µg Pot^{-1}) by Paddy at Flowering

Sources of Organic Waste	Levels of organic waste		Mean
	1.5%	**3.0%**	
Sewage sludge	6.06	6.85	6.45
Tannery waste	4.14	3.25	3.70
Mean	5.10	5.05	

Control: 4.11 (µg pot^{-1})

	SOW	L	SOW x L
S.Em.(±)	0.055	0.064	0.079
C.D. (5%)	0.16	N.S.	0.23

Table 6b Interaction Effect of Sources of Organic Waste And Amendments on Chromium Uptake (µg Pot^{-1}) By Paddy at Flowering

Sources of organic waste	No amendment	Amendments		Mean
		$CaCO_3$	EDTA	
Sewage sludge	9.48	6.15	3.73	6.45
Tannery waste	3.26	4.32	3.50	3.70
Mean	6.37	5.24	3.62	

	SOW	A	SOW x A
S.Em.(±)	0.055	0.069	0.097
C.D. (5%)	0.16	0.20	0.28

Nickel Concentration in Paddy

The data presented in table 7 clearly indicated that all the treatments of tannery waste *i.e.* from T_8 to T_{13} exhibited significantly higher Ni concentration in plants in comparison to their corresponding treatments of sewage sludge *i.e.* from T_2 to T_7. The Ni content in plants among the treatments based on tannery waste varied from 1.15 µg g^{-1} with T_8 to 4.43 µg g^{-1} with T_{13}, while among the sewage sludge-based treatments, Ni concentration in plants ranged from 0.82 µg g^{-1} with T_2 to 1.40 µg g^{-1} with T_7 as against 0.72 µg g^{-1} with control (T_1). The influence of EDTA on Ni content in plants in comparison to $CaCO_3$ both used as amendments in combination with the levels of organic wastes was visibly higher with tannery waste.

Data on the effect of organic wastes, their levels, amendments and their interactions on Ni concentration in paddy plants at flowering have been presented in interaction table 8a and 8 b.

. It is obvious from the data shown in interaction table 8a that between the two sources of organic wastes the application of tannery waste resulted in significantly higher mean Ni concentration in paddy plants (2.28 µg g^{-1}) in comparison to sewage sludge (1.02 µg g^{-1}). Out of two levels, 3.0% level caused significantly greater Ni content in plants (1.80 µg g^{-1}) over 1.5% level (1.5 µg g^{-1}). The interaction between the levels and sources of organic waste was significant in respect of Ni content in plants. The application of tannery waste at 1.5% level (2.06 µg g^{-1}) was better than the application of sewage sludge at 3.0% level (1.10 µg g^{-1}) so far as the Ni concentration in plants was concerned.

From the data shown in interaction table 8b it was evidently clear that between the two amendments *i.e.* $CaCO_3$ and EDTA the influence of EDTA, (2.60 µg g^{-1}) was significantly more than that of $CaCO_3$ (1.30 µg g^{-1}) with respect to mean Ni concentration in plants. The interaction effect of organic wastes and amendments was significant in this respect.

Table 7 Effect of Various Treatments Of Organic Wastes On Nickel Concentration In Paddy At Flowering

Code no.	**Ni concentration in paddy (µg g^{-1})**
T_1	0.72
T_2	0.82
T_3	0.92
T_4	1.10
T_5	0.90
T_6	1.00
T_7	1.40
T_8	1.15
T_9	1.55
T_{10}	3.47
T_{11}	1.30
T_{12}	1.70
T_{13}	4.43

S.Em.(±)	0.08	0.38
C.D. (5%)	0.23	1.31

Table 8a. Interaction effect of levels and organic wastes on nickel concentration (µg g^{-1}) in paddy at flowering

Sources of Organic Waste	Levels of organic waste		Mean
	1.5%	3.0%	
Sewage sludge	0.947	1.100	1.023
Tannery waste	2.057	2.494	2.276
Mean	1.502	1.797	

Control: 0.72 (µg g^{-1})

	SOW	L	SOW **x** L
S.Em.(±)	0.033	0.033	0.046
C.D. (5%)	0.096	0.096	0.135

Table 8b Interaction Effect of Sources of Organic Waste And Amendments on Nickel Concentration (µg G^{-1}) in Paddy at Flowering

Sources of organic waste	No amendment	Amendments		Mean
		$CaCO_3$	EDTA	
Sewage sludge	0.860	0.960	1.250	1.023
Tannery waste	1.225	1.650	3.950	2.276
Mean	1.043	1.305	2.601	

	SOW	A	SOW **x** A
S.Em.(±)	0.033	0.040	0.057
C.D. (5%)	0.096	0.117	0.166

Nickel Uptake in Paddy

A close examination of data shown in table 9 clearly indicated that various treatments of two organic waste except the treatment T_{11} significantly enhanced Ni uptake in paddy at flowering over control. The treatments based on sewage sludge (T_2, T_3, T_5 and T_6) barring the treatments T_4 and T_7 exhibited significantly higher values for Ni uptake in paddy in comparison to their corresponding treatments based on tannery waste (T_8,T_9, T_{11} and T_{12}). The treatments T_{10} and T_{13} where tannery waste was used in combination with EDTA had significantly higher values for Ni uptake in paddy over their corresponding treatments (T_4 and T_7) based on sewage sludge. Nickel uptake in paddy at flowering across the treatments varied from 6.46 µg pot^{-1} with T_{11} to 12.74 µg pot^{-1} with T_{10} as against 5.92 µg pot^{-1} observed with control (T_1).

Data on Ni uptake by paddy at flowering as affected by various factors such as sources of organic waste, their levels, amendments and their interactions have been demonstrated in interactions table 10a,b and Fig.2.

Data shown in interaction table 10a reveals that out of the two sources of organic waste studied, the sewage sludge (9.65 µg pot^{-1}) significantly increased Ni uptake in paddy over tannery waste (9.07 µg pot^{-1}). The effect of another main factor *i.e.* levels of organic waste (1.5 and 3.0%) was not significant so far as Ni uptake in paddy was concerned. The interaction effect between the levels and sources of organic waste was also not significant in relation to Ni uptake in paddy (table 10a).

It is obvious from the observation presented in interaction table 24b that Among the amendments *viz.* $CaCO_3$, EDTA and no amendment tried, the influence of EDTA in enhancing Ni uptake in paddy was significantly more as compared to $CaCO_3$ and no amendment. The corresponding values for Ni uptake in paddy were 9.19, 9.71 and 9.18 µg pot^{-1}, respectively. The interaction between sources of organic waste and amendments was significant with respect to Ni uptake in paddy. The application of tannery waste without any amendment (7.17 µg pot^{-1}) proved as effective as the application of sewage sludge in combination with EDTA (7.53 µg pot^{-1}) in relation to the uptake of Ni in paddy (Table 10b). The effect of organic waste in this connection has already been covered in previous table 10a.

Table 9 Effect of Various Treatments of Organic Wastes on Nickel Uptake In Paddy At Flowering

Code no.	Ni uptake in paddy (µg pot^{-1})
T_1	5.92
T_2	10.03
T_3	10.45
T_4	7.35
T_5	12.33
T_6	10.05
T_7	7.71
T_8	7.89
T_9	8.94
T_{10}	12.74
T_{11}	6.46
T_{12}	7.33
T_{13}	11.04
S.Em.(±)	0.38
C.D. (5%)	1.13

Table 10a Interaction Effect of Levels and Sources of Organic Waste on Nickel Uptake (µg Pot^{-1}) In Paddy at Flowering

Sources of organic waste	Levels of organic waste		Mean
	1.5%	3.0%	
Sewage sludge	9.28	10.03	9.65
Tannery waste	9.86	8.28	9.07
Mean	9.57	9.15	

Control: 5.92 (µg pot^{-1})

	SOW	L	SOW x L
S.Em.(±)	0.166	0.166	0.196
C.D. (5%)	0.48	N.S.	N.S.

Table 10b. Interaction Effect of Sources of Organic Waste and Amendments on Nickel Uptak (µg Pot^{-1}) In Paddy at Flowering

Sources of organic waste	No Amendment	Amendments		Mean
		$CaCO_3$	EDTA	
Sewage sludge	11.18	10.25	7.53	9.65
Tannery waste	7.17	8.14	11.89	9.07
Mean	9.18	9.19	9.71	

	SOW	A	SOW x A
S.Em.(±)	0.166	0.204	0.288
C.D. (5%)	0.48	0.59	0.83

Lead Concentration in Paddy

A critical appraisal of data demonstrated in table 11 reveals that application of two sources of organic waste significantly influenced the Pb concentration in paddy plants. The Pb content in plants among the sewage sludge based treatments ranged from 1.10 µg g^{-1} with T_8 to 1.96 µg g^{-1} with T_{13} as against 0.98 µg g^{-1} obtained with control (T_1). It was further noticed that sewage sludge or tannery waste at 3.0 % level applied in combination with $CaCO_3$ and EDTA as amendment had in general significantly higher Pb concentration in plants over their corresponding treatments of amendments applied in combination with 1.5% level of either organic waste.

Data pertaining to the influence of individual factors such as organic wastes, their levels, amendments and their interactions on Pb concentration in paddy at flowering have been presented in table 12a and 12 b.

Data shown in interaction table 12a clearly indicated that main factors of levels and sources of organic wastes significantly influenced the Pb concentration in plants at flowering. Between the two sources of organic wastes, the tannery waste (1.61 µg g^{-1}) produced significantly higher mean Pb content in plants over sewage sludge (1.46 µg g^{-1}). Similarly, out of the two levels of organic wastes, the 3.0% level (1.74 µg g^{-1}) caused significantly greater Pb content in plants in comparison to 1.5% level (1.34 µg g^{-1}). The interaction between levels and sources of organic waste was significant with respect to Pb content in plants.

It is apparently clear from the data presented in interaction table 12b that $CaCO_3$ and EDTA tried as amendments could not bring about any significant change in Pb concentration in plants. The effect of sources of organic waste on Pb content in plants has already been discussed in previous interaction table 12a. The interaction between amendments and sources of organic waste was not significant in relation to Pb content in plants.

Table 11 Effect of Various Treatments of Organic Wastes on Lead Concentration in Paddy at Flowering

Code no.	Pb concentration in paddy (µg g^{-1})
T_1	0.98
T_2	1.10
T_3	1.25
T_4	1.38
T_5	1.45
T_6	1.76
T_7	1.83
T_8	1.21
T_9	1.41
T_{10}	1.67
T_{11}	1.59
T_{12}	1.84
T_{13}	1.96

S.Em.(±)	0.05
C.D.(5%)	0.15

Table 12a Interaction Effect of Levels and Sources of Organic Waste on Lead Concentration (µg G^{-1}) in Paddy at Flowering

Sources of organic waste	Levels of Organic waste		Mean
	1.5%	3.0%	
Sewage sludge	1.243	1.682	1.463
Tannery waste	1.430	1.796	1.613
Mean	1.337	1.739	

Control: 0.98 (µg g^{-1})

	SOW	L	SOW **x** L
S.Em.(±)	0.022	0.022	0.031
C.D. (5%)	0.064	0.064	0.091

Table 12b Interaction Effect of Sources of Organic Waste and Amendments on Lead Concentration (µg G^{-1}) In Paddy at Flowering

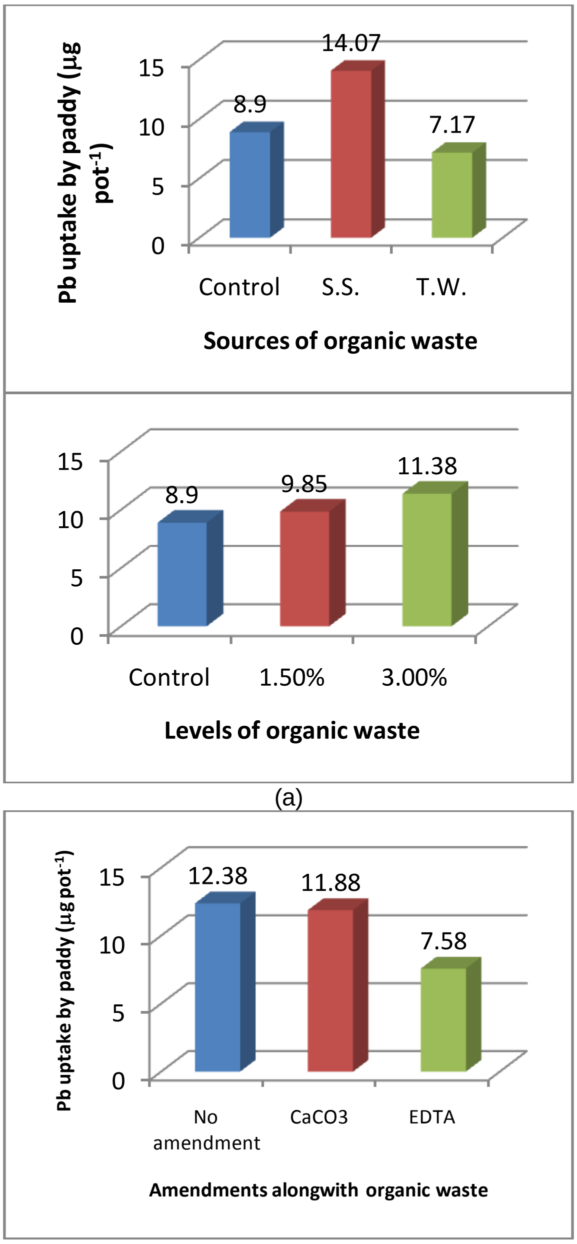
Sources of organic waste	No amendment	Amendments		Mean
		$CaCO_3$	EDTA	
Sewage sludge	1.275	1.507	1.607	1.463
Tannery waste	1.398	1.625	1.815	1.613
Mean	1.337	1.566	1.711	

	SOW	A	SOW **x** A
S.Em.(±)	0.022	0.022	0.039
C.D. (5%)	0.064	N.S.	N.S.

Lead Uptake In Paddy

A critical appraisal of data on Pb uptake in paddy as shown in table 13 reveals that the different treatments based on sewage sludge barring the treatments T_4 significantly increased the Pb uptake in paddy at flowering over control (T_1) as well as the treatments based on tannery waste. The treatments having EDTA either in combination with sewage sludge or tannery waste (T_4, T_7, T_{10} and T_{13}) showed invariably significantly lower Pb uptake values in paddy in comparison to the rest of the treatments of respective waste category. All the treatments of sewage sludge exhibited a variation in Pb uptake values in paddy ranging from 9.23 µg pot^{-1} with T_4 to 19.96 µg pot^{-1} with T_5 while a variation ranged from 4.88 µg pot^{-1} with T_{13} to 8.19 µg pot^{-1} with T_8 and T_9 across the treatments based on tannery waste as against 8.10 µg pot^{-1} noticed with control (T_1).

Data on Pb uptake by paddy at flowering as affected by various factors such as sources of organic waste, their levels, amendments and their interactions have been demonstrated in interaction table 14a,b and Fig. 3.



(a)

(b)

Fig. 3: (a) Effect of sources and levels of organic waste and (b) amendments on mean Pb uptake by paddy at flowering

It is evidently clear from the data on Pb uptake in paddy at flowering shown in interaction table 14a that of the two organic wastes tested, the sewage sludge caused significantly higher Pb uptake in paddy as compared to tannery waste. The mean Pb uptake value was as high as 14.07 µg pot^{-1} observed with sewage sludge and as low as 7.17 µg pot^{-1} noticed with tannery waste. Between the two levels of organic waste, 3.0% level (11.38 µg pot^{-1}) resulted significantly higher Pb uptake in paddy in comparison to 1.5% level (9.85 µg pot^{-1}). The interaction between the levels and sources of organic waste was significant in this respect (Table 14a).

It is obvious from the data on Pb uptake in paddy presented in interaction table 14b that both CaCO$_3$ (11.88 µg pot^{-1}) and no amendment (12.38 µg pot^{-1}) were comparable to each other and showed significantly higher mean Pb uptake values over EDTA (7.58 µg pot^{-1}). The interaction between the sources of organic waste and amendments was significant in respect of Pb uptake in paddy. The main effect of organic waste in this connection has already been discussed in previous table 13.

Table 13 Effect of Various Treatments Of Organic Wastes On Lead Uptake In Paddy At Flowering

Code no.	Pb uptake in paddy (µg pot^{-1})
T$_1$	8.10
T$_2$	13.49
T$_3$	13.84
T$_4$	9.23
T$_5$	19.96
T$_6$	17.78
T$_7$	10.09
T$_8$	8.19
T$_9$	8.19
T$_{10}$	6.14
T$_{11}$	7.88
T$_{12}$	7.72
T$_{13}$	4.88

S.Em.(±)	0.44
C.D. (5%)	1.30

Table 14a Interaction Effect of Levels and Sources of Organic Waste on Lead Uptake (µg Pot^{-1}) In Paddy at Flowering

Sources of organic waste	Levels of organic waste		Mean
	1.5%	**3.0%**	
Sewage sludge	12.19	15.95	14.07
Tannery waste	7.51	6.82	7.17
Mean	9.85	11.38	

Control: 8.10 (µg pot^{-1})

	SOW	L	SOW **x** L
S.Em.(±)	0.184	0.184	0.263
C.D.(5%)	0.53	0.53	0.76

Table 14b Interaction Effect of Sources of Organic Waste And Amendments On Lead Uptake (µg Pot^{-1}) In Paddy At Flowering

Sources of organic waste	No amendment	Amendments		Mean
		CaCO$_3$	EDTA	
Sewage sludge	16.73	15.81	9.66	14.07
Tannery waste	8.03	7.95	5.51	7.17
Mean	12.38	11.88	7.58	

	SOW	A	SOW x A
S.Em.(±)	0.184	0.225	0.320
C.D. (5%)	0.53	0.65	0.93

Discussion

The sewage sludge-amended soil produced a significant rise to the tune of 19.52% whereas tannery waste-amended soil caused a sharp decline to the tune of 111.96% in mean drymatter yield of paddy at flowering over control. The beneficial effect of sewage sludge-treated soil, in contrast to, the adverse effect of tannery waste-treated soil on the biomass production of paddy at flowering may be ascribed mainly to the considerable variation in mineral and organic composition of two organic wastes, the transformation of plant nutrients element and heavy metals as well as the formation of harmful inorganic and organic reduction products during the anaerobic decomposition of organic matter, and physical, chemical, physicochemical and biological environment of soil prevailed under the continuous submergence.

The submerged soil undergoes reduction (oxygen is depleted) and soil microorganism then use oxidised soil components as electron acceptors in their respiration. The components are reduced in the following order: nitrate, manganese dioxide, ferric hydroxide, sulphate and carbondioxide (Ponnamperuma, 1972). As nitrate disappear, large amounts of Mn, and Fe enter the soil solution, and sulphate is converted to insoluble sulphides. Thus, soil reduction eliminates NO_3, increases the concentration of water soluble Mn, and Fe, changes the iron to Mn ratio in soil solution and lowers the availability of sulphate. The secondary effect of soil reduction include an increase in the solubility and availability of phosphate and silica to rice (Ponnamperuma, 1965). Organic matter decomposes anaerobically producing variety of substances, of which CO_2, organic acids (mainly acetic and butyric with small amounts of formic, propionic and lactic acids), methane, H_2S and mercaptans *etc*. are the most important (Pannamperuma *et al,. 1966*).

Sewage sludge had semi-decomposed organic matter (24.8% C), very rich in Fe (14.3mg g^{-1}) along with high concentration of other plant-micronutrients and appreciable concentration of heavy metal elements, where as tannery waste contained higher amount of organic matter(35.2% C) with considerable proportion of proteinaceous, gelatinous and lipid substances removed from raw hide / skin of animals during cleaning and tanning process, slowly decomposable, very rich in Cr content (364 µg g^{-1}) along with high concentration of total N and sulphate (2.35%). In sewage sludge – amended soil, high in iron, H_2S was rendered harmless by precipitation as ferrous sulphide and could not accumulate in the root zone , thus root development, nutrient uptake and plant growth were not affected adversely , resulting in higher drymatter production in paddy at flowering. Against this , in tannery waste-amended soil being high in sulphate, organic matter , Cr content and low in iron, the H_2S and organic acids were generated in high concentration which directly interferred the root development and nutrient uptake or poisoned the rice plants. Moreover, possibly Cr accumulation in high concentration in roots might be aggravated the effect of harmful substances on plant growth. All these factors , concomittantly resulted in drastic decline in biomass production of paddy over control.

Mitsui *et al.* (1951,1959) demonstrated that a H_2S concentration as low as 0.07 ppm and organic acids concentrations of 10^{-2}-10^{-3} M are toxic to rice . Cr toxicity has been reported for vegetable crops grown in tannery sludge-amended soils (Sara Parwin Banu *et al*.,2000).

Further, increasing levels of organic wastes (1.5 and 3.0%) resulted in gradual depression in mean drymatter yield of paddy at flowering , being significant at 3.0 % level over control. Greenhouse plant growth studies using tannery sludge- treated soils have shown toxicity of Cr when sludge was applied at a rate exceeding 3g kg^{-1} and a significant yield reduction was also observed (Naidu *et al*.,2000). Soil incubation with 3.0% rate of organic waste enriched the soil with double amount each of plant nutrients, heavy metals and organic substances. Sulphate reduction and anaerobic decomposition of organic matter in soil produced toxic substances (H_2S various organic acids and organic reduction products) which adversely affected the root proliferation and development, interferred the nutrient uptake and restricted the plant growth. The plants could not get the benefit of increased concentration and availability of plant nutrients. This reason led to the significant depression in biomass yield at 3.0 % level of organic waste. Sewage sludge at 3.0% level elevated the drymatter production of paddy at flowering by 13.49 %, while tannery waste even at 1.5 % level declined the drymatter yield by 53.56% over control. This clearly indicated that anaerobic decomposition of tannery waste's constituents even at lower level (1.5%) generated the harmful substances in concentration deterimental to the normal growth of paddy plants, in addition to the decreased availability of micronutrients to plants due to their sulphide formation. The results from the present study corroborate the findings of Sara Parwin Banu *et al.* (2000) and Ponnamperuma *et al.* (1976).

Addition of both $CaCO_3$ and EDTA along with organic waste declined the drymatter yield of paddy by 21.63 and 106%, respectively. Calcium carbonate decreased the solubility and availability of micronutrients elements to plants by the formation of their insoluble carbonates and hydroxides, thus influenced the plant growth adversely. As a result, the drymatter yield was depleted. EDTA formed the soluble metals chelate, which were taken up by plants, accumulating their larger fraction in root and smaller fraction in shoot. However, chelated metals concentration in soil solution elevated to a level (as was evidenced by the observation on DTPA extractable fraction of metals in soil noticed with EDTA treatment), at which further deposition in excess proportion in roots and translocation to shoots caused harmful effect on plant gowth and thus drastically cut down the drymatter yield.

Huffman and Allaway (1973) have reported as much as 98% chromium accumulation in the roots of bean plants. The reason for the high accumulation in roots of the plant could be because chromium is immobilised in the vacuoles of the root cells (Shanker *et al;* 2004). In solution culture studies with paddy plants Cr (III) and Cr (VI) behaved similarly and 90% of Cr was retained by roots and only about 1% was translocated to leaves (Mishra et al., 1997). Plants growing in soil amended with Cr (III) salts, chromium hydroxides and sewage sludge were found to contain very small amount of Cr particularly in the aerial parts of the plant and the uptake has been reported to be significant only in roots (Boulton, 1975, Lahouti and Peterson, 1979). The mean Cr, Ni and Pb concentration in paddy plants were significantly higher with tannery waste-treated soil as compared to with sewage sludge-treated soil. The plant concentration of these metals with sewage sludge and tannery waste amended soil was in the order as Pb>Ni>Cr and Ni>Pb>Cr, respectively, while the uptake of these heavy metals, in contrast to plant concentration was 1.74 time higher for Cr, significantly higher for Ni and almost two time higher for Pb with sewage sludge-amended soil as compared with tannery waste-amended soil.

Significantly lower concentration of heavy metals in plants with sewage sludge-amended soil may be attributed mainly to the dilution effect of enhanced drymatter production with sewage sludge treated soil. The uptake parameter is essentially governed by the metal concentration in plants and biomass yield of the crop., consequently, the higher uptake of heavy metals by paddy with sewage sludge-amended soil was the resultant product of heavy metal content in plants and drymatter yield.

The mean heavy metal uptake by the crop with sewage sludge and tannery waste-amended soils followed the trend similar to metal concentration in plants which was Pb>Ni>Cr and Ni>Pb>Cr, respectively. In sewage sludge-amended soils, very stable organic complexes account for the extremely low availability of the element. However, complexing Cr (III) with soluble organic acids (*e.g.* citric acid, DTPA, fulvic acids and soil extracts of water-soluble organic matter) maintain Cr (III) in solution above the pH at which uncomplexed Cr precipitates and is therefore a means of enhancing its mobility (James and Bartlett, 1983). Uptake of Cr by plants and transport to the aerial tissue is minimal at near-neutral soil, characteristic of Cr (III) (Davies and Wixon, 1985).

Tannery waste applied even at 1.5% level produced significantly higher Cr and Ni concentration in paddy plants than the sewage sludge applied at 3.0% level. The adequate availability of Cr from the Cr rich-tannery waste applied at 1.5% might be the possible reason for higher concentration of Cr in plants.

Increasing levels of organic wastes brought about significant rise in mean concentration of Cr, Ni and Pb in paddy plants at flowering. A significant increase in mean concentration of heavy metals in plants resulted due to the graded increase in the levels of organic waste. Such increase in metal concentration in plants is expected due to the progressively increased proportion of heavy metals (Cr, Ni and Pb) supplied through increasing levels of organic waste and consequent elevated release and availability of metals to plants. Enhanced levels of organic wastes could not bring about any significant change in Cr and Ni uptake but produced significant rise in Pb uptake by paddy. The higher Pb uptake may be ascribed to the significantly higher Pb concentration in plants due to the incorporation of organic wastes at 3.0% rate.

Both the amendments, $CaCO_3$ and EDTA significantly elevated the metals concentration in paddy plants but significantly declined the Cr and Pb uptake over no amendment. However, the effect of EDTA was more pronounced in this connection.

EDTA being a complexing agent, formed soluble metals chelate of fairly high stability constants at nearly neutral soil pH. EDTA facilitated the accumulation of micronutrients elements and heavy metals in roots and their subsequent translocation to the aerial portion of the plant, thereby resulting in higher concentration of metals in plants. Cataldo *et al*. (1978) stated that Ni supplied as an EDTA complex enhances water solubility and plant accumulation of the element. Within the plant, Ni is considered to be a highly mobile element. In soyabean, during vegetative growth Ni was found to accumulate mainly in leaves, but during senescence a considerable fraction was mobilised to seeds. Ensley *et al*., (1999) maintain that chelating agents (such as EDTA) and acids increase both solubility of metals in the soil and root to shoot transfer of metals. This transfer is due to disrupting the plant metabolism that regulates the transport of metal to the shoots.

The synergistic and antagonistic interactions of plant nutrients element and heavy metals operating in soil-plant system further influence the EDTA mediated uptake mechanism of plant, rendering the plant too deficient or too sufficient in one or two metals and affecting the plant growth adversely, thereby resulting a reduction in Cr and Pb uptake and drymatter yield. Furthermore, the concentration of harmful products such as H_2S, various organic acids and other organic reduction products formed under anaerobic conditions may also aggravate the effect of deficiency or sufficiency of metals on plant growth and metals uptake.

References

Anderson, C.W.N., Brooks, R.R., Stewart, R.B. and Simcock, R. (1998). Harvesting a crop of gold in plants. Nature. 395: 553-554.

Bhatia, M., Babu, R.S., Sonawane, S.H., Gogate, P.R., Girdhar, A., Reddy, E.R. and Pola, M. (2016). Application of nanoadsorbents for removal of lead from water. Intl. J. Environ. Sci. Technol. doi:10.1007/s13762- 016-1198-6

Blaylock, M.J. (2000). Field demonstration of phytoremediation of lead contaminated soils. In N. Terry and G.Banueloz (ed.) phytoremediation of contaminated soil and water. Lewis-Publ. Boaca Raton. Florida. p. 1-12.

Blaylock, M.J., Salt, D.E., Dushenkov, S., Zakharova, O., Gussman, C., Kapulnik, Y., Ensley, D.B. and Raskin, I. (1997). Enhanced accumulation of Pb in Indian mustard by soil-applied chelating agents. Environ Sci. Technol. 31: 860-865.

Boulton, J. (1975). Liming effect on the toxicity to perennial ryegrass for a sewage sludge *contaminated with zinc, nickel, copper and chromium. Environ. Pollu. 9: 295-304.*

Cataldo, D.A., Garland, T.R. and Wildung, R.E. (1978). Nickel in plants. I. Uptake kinetics using intach soybean seeding. Plant Physiol. 62: 5636-5665.

Cooper, E.M., Sims, J.T, Cunnigham, S.D., Huany, J.W. and Berti, W.R. (1999).

Chelteassisted phytoextraction of lead from contaminated soils. J. Environ. dual. 28: 1709-19.

Davies, B.E. and Wixon, B.G. (1985). Trace elements in surface soils. J. Soil Sci. 36: 551-570.

Dhongade, S. and Nandkar, P.B. (2011). The phytotoxicity of heavy metals on growth and metal uptake by spinach. Agr. Biol. Res. 27: 78–90

Ensley,B.D., Blaylock,M.J., Dushenkov, S., Kumar,N.P.B.A. and Kapulnik, Y. (1999). Inducing hyeracumulation of metals in plant shoots. U.S. Patent 5917117.

Giridhara, M. and Siddaramappa, R. (2002). Effect of heavy metals on urease activity in soil. Curr. Res. Univ. Argric. Sci. Bangalore31: 4–5.

Huang, J.W., Blaylock, M.J., Kapulnik, Y. and Ensley, B.D. (1998). Phytoremediation of uranium contaminated soil: Role of organic acids in triggering uranium hyperaccumulation in plants. Environ Sci. Technol. 32: 2004-2008.

Huang, J.W., Chen, J., Berti, W.B. and Cunningham, S.D. (1997). Phytoremediation of lead contaminated soil: Role of synthetic chelates in lead Phytoextraction. Environ. Sci. Technol. 31: 800-805.

Huang, J.W. and Cunnigham, S.D. (1996). Lead phytoextraction species variation in lead uptake and translocation. New Phytol. 13: 75-84.

Huffman, E.W.D. and Alloway, W.H. (1973). Chromium in plant : distribution in issue. Organelles and extracts and availability of bean leaf chromium to animal. J. Agr. Chem. 21: 282-986.

James, BR. and Bartlett, R.J. (1983). Behavior of chromium in soil VII. Adsorption and reduction of hexavalent forms. J. of Enviorn. Qual. 12: 177-181.

Kalaivanan, D., Ganeshamurthy, A.N. (2016). Mechanism of heavy metal toxicity in plants. Abiotic stress physiology of horticultural crops. DOI 10.1007/978-81-322-2725-0_5

Khan, S., Aijun, L., Zhang, S., Hu, Q. and Zhu, Y.G. (2008). Accumulation of polycyclic aromatic hydrocarbons and heavy metals in lettuce grown in the soils contaminated with long-term wastewater irrigation. J. Hazard. Mater. 152: 506–515

Kucharski, J., Wyszkowska, J. (2004). Inter-relationship between number of microorganisms and spring barley yield and degree of soil contamination with copper. Plant Soil Environ.50: 243–249.

Lasat, M.M., Baker, A.J.M. and Kochian, L.V. (1998). Altered Zinc compartmentation in the root symplasm and stimulated Zn^{+2} absorption into the leaf as mechanisms involved in Zinc hyper-accumulation in Thlaspi caerulescens. Plant Physiol. 118: 875-883.

Lahouti and Peterson. (1979). Chromium accumulation and distribution in crop plants. J. Sci. Food Agric. 30: 136-142.

Liu, W.J., Zhu, Y.G., Smith, F.A. and Smith, S.E. (2004). Do iron plaque and genotypes affect arsenate uptake and translocation by rice? J. Exp. Bot.55: 1707–1713

Mishra, S., Sanker, K., Srivastava, M. M., Srivastava, S., Shrivastav, R., Dass, S. and Prakash, S. (1997). A study on the uptake of trivalent and hexavalent chromium by paddy (oryza Sativa). Possible chemical modifications in rhizospere. Agric Ecosyst Environ. 62: 53-58.

Mitsui, S., Aso, S. and Kumazawa, O.K. (1951). Dynamic studies on the nutrient uptake by crop plants. I. The nutrient uptake of rice root as influence by hydrogen sulfide (In Japanese English Summary). J. Sci. Soil Manure. Jpn. 22: 46-52.

Mitsui, S., Kumazawa, K. and Hishida.T. (1959). Dynamic studies on the nutrient uptake by crop plants.23. The growth of rice plant in poorly drained soil as affected by the accumulation of volatile organic acids 2 (In Japanese). J. Sci. Soil Manure. Jpn. 30: 411-413.

Naidu, P.H.(2000). Respones of bunch varieties of groundnut to Rhizobium inovulation. Legume Research. 23:130-132.

Pandey, N. and Sharma, C.P. (2002). Effect of heavy metals Co2+, Ni2+ and Cd2+ on growth and metabolism of cabbage. Plant Sci.163: 753–758

Ponnamperuma, F.N. (1965). Dynamic aspects of flooded Soils. Pages 295-328 in International Rice Research Institute. The mineral nutrition of the rice plant. Proceedings of a symbosium at the Internation Rice Research Institute. The Johns Hopkins Press, Baltmore, Maryland.

Ponnamperum, F.N., Martinez, E. and Loy, T. (1966). Ingluence of redox potential and partial pressure of carbon dioxide on the pH value and the suspension effect of flooded soils. Soil Sci. 101: 421-431.

Ponnamperuma, F. N. (1972). The chemistry of submerged soils. Adv. Agron. 24: 29-96.

Sara Prawin Banu, K., Ramesh, P. T., Ramasamy, K., Mahimairajah, S. and Naidu, R.(2000). Is it safe to use tannery chrome sludge for growing vegetables? Result from a glasshouse study. In: Naidu, R., Willets, I. R., Mahimairaja, S., Kookana, R. and Ramasamy, K.(eds.). Towards Better Management of Soil Contaminated with Tannery waste. ACIAR Proceedings no 88. Canberra, Australia. p. 127-132.

Shanker A.K., Djanaguiraman, M., sudhagar, R., Chandrasheker, C.N. and Pathmanabhan G. (2004).Differential antioxidative response of ascorabte glutathione pathway enymes and metabolities to chromium speciation stress in green gram (Vigna Radiata (L) R Wilczek, CV (04) roots. Plant Sci. 166: 1035-43.

Wyszkowska, J., Kurcharski, J. and Lajszner, W. (2006). The effects of copper on soil biochemical properties and its interaction with other heavy metals. Polish J. Environ. Studies15:927–934.

Wyszkowska,J.,Boros, E. and Kucharski, J. (2007). Effect of interactions between nickel and other heavy metals on the soil microbiological properties. Plant Soil Environ.53 (12): 544–552

Yizong, H., Ying, H. and Yunxia, L. (2009). Combined toxicity of copper and cadmium to six rice genotypes (Oryza sativa L.). J. Environ. Sci. 21: 647–653.