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Adsorbent Potential of Tea Waste to Control Cadmium Toxicity on Wheat (*Triticum aestivum* L) Seedling Growth

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ABSTRACT

The disposal of industrial wastage without proper treatment is responsible for the lowering of crop productivity with the accumulation of essential and non essential trace metals in the land. The present research was designed to evaluate Cadmium toxicity on plant growth and to describe the remedial effect of tea wastage against Cd(II) toxicity with reference to the growth of wheat (*Triticum aestivum* L).Application of Cd^{2+} decreased the wheat seedling growth along with alleviated concentration. It was dose-dependent, and significant at higher concentration of $CdCl_2$. The result showed the inhibitory effect of Cd^{2+} ion on plant growth which includes reduction in shoot and root length, plant fresh and dry biomass and soluble carbohydrate and significant increase in total phenol contents as defense biomolecule against external stress. Adsorption is a promising alternative method to treat industrial effluents. Mainly because of its low cost and high metal binding capacity tea waste is one of the low cost and easily available adsorbent having strong adsorptivity towards heavy metals. The consumed tea leaves were found to be able to remove substantial amounts of Cd^{+2}ions from aqueous solution. Thus it can be inferred that the addition of tea waste at appropriate rate may be useful approach to enhance the plant growth and to immobilize Cd^{2+} by depressing its bioavailability.

Keywords: toxic metals, remedial effect, tea wastage, bioavailability

1. INTRODUCTION

Cd^{2+} is recognized as an extremely significant pollutant due to its high toxicity and large solubility in water[1].Cadmium can alter the uptake of minerals by plants through its effects on minerals availability[2]. Stomatal opening, transpiration, and photosynthesis[3] have been reported to be affected by Cd^{2+}. Like other divalent ions, Cd^{2+} is also able to displace essential metal cations from proteins generally leading to inhibition of enzyme activities. Among other effects, Cd^{2+} generates free radicals and reactive oxygen species (ROS) in plant and animal tissues, leading to oxidative stress [4, 5]. Adsorption is a promising alternative method to treat metal ions effects. Mainly because of metal binding capacity[6,7], tea waste is one of the low cost and easily available adsorbent having strong adsorptivity towards metals like Cd, Zn, Ni, and Pb[8,9]because of the soft colloid and chemical components like palmatic acid of fatty group and terpenes present in it [10].In the given research, the adsorption ability of tea waste was investigated for the removal of Cd(II) from aqueous systems. It has been suggested that the metal uptake capacity of tea waste is due to the chelating properties of its constituents especially lignin and cellulose. These materials are the major constituents of cell walls and possess polar functional groups such as alcohols, aldehydes, ketones, carboxylic acids, Phenolic hydroxides and ethers. The polar nature of these complex materials can remove large quantities of metal ions from solutions [11].

2. MATERIAL AND METHOD

A set of treatments was designed with different levels of $CdCl_2$ concentrations alone and along with tea waste @5ton/ha (i.e.2.25gm/lit). The experiment was laid out in randomized complete block design (RCBD) in plate and pot culture having three replicates of each treatment. For the preparation of aqueous extract a well dried consumed tea waste were dispersed in distilled water for overnight, the extract was then filtered and stored at cool place. Research grade $CdCl_2$ was used to prepare 1000ppm Cd stock solution in deionized double distilled water and dilution were made from 5ppm-1000ppm carefully.

3. EXPERIMENTAL SETUP

Ten healthy sterilized seed of wheat (*Triticum aestivum L*) were placed in Petri dishes with one disc of filter paper under normal laboratory condition at 21-25^{0}C. Five ml of each treatment were added to each treatment plate. Distilled water was applied to the control level. The growth parameters including length and weight were recorded after 6th day of germination. For the analysis of some useful biochemical parameters, a set of 50ppm, 100ppm and 150ppm Cd, alone and along with tea waste was established on soil medium. After a month of treatment exposure, plants were harvested and plants were subjected to the analysis of biochemical test to check the toxic effect of cadmium on these processes of plant. Soluble Carbohydrate was estimated by Yemm & Willi's method, 1954 [12]and Total Phenol Estimation by Ainsworth & Gillespie, 2007[13].

3.1. *Inhibitory Percentage* (I)

The % of inhibition over control was calculated by Surendra and Pota, 1978[14] formula: I = 100-T/C x 100. Where, I is the % of inhibition, T is treatment reading and C is control reading.

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3.2 *Statistical Analysis*

The data obtained was statistically analyzed and presented as ± SE and Standard deviation calculated by SPSS programme to examine the differences between each treatment. The level of statistical significance was set at $P \geq 0.05$.

4. RESULT AND DISCUSSION

Cadmium toxic effect on plant growth was examined in terms of seedling height, biomass and biochemical production. The result showed that the elevated Cd concentration (i.e. 5ppm -1000ppm) significantly increased the toxic effect of heavy metal on Shoot and Root length, fresh and dry weight of wheat (*Triticum aestivum*) seedling. These observations are in accordance with the findings of Hsu and Chang-Hung Chou [15], who determined the inhibitory effects of some heavy metals ($CdCl_2$, $CuS0_4$, $PbCl_2$, $HgCl_2$) on *Miscanthus* species. Rubio *et al.*[16]also reported that, length and dry matter accumulation were significantly lower in Cd-treated rice *(Oryza sativa* L.) plants than in the control. The given results also notify that, the plant response to Cd treatment was strongly dependent on concentration at which $CdCl_2$ was applied. These results confirmed the report of Zaman and Zereen[17], who reported a dose-related growth inhibition in the radish *(Raphanus sativus* L.) in the presence of Cd. At 20-40ppm, the shoot and root length of wheat seedling showed tolerance against the Cd toxicity proving the safe level as described as USEPA[18]guideline.This increase in growth parameters at low concentration of Cd could be due to the presence of the phenomenon of *hormesis*, a response of the seedlings where the low dose stimulates the growth while high dose suppresses the growth [19]. In the given research, 5ppmCd–40ppmCd levels were found beneficent for shoot length and less inhibitory for plant dry weight accumulation, this may be due to the conservative strategy of crop to survive in an stressed environment, that may resulted in increased dry weight, due to accumulation of primary product of plant under stress condition. Although, all the high doses of Cd^{2+} greatly influenced dry weight accumulation. Due to the biosorbent property of tea waste, when applied with $CdCl_2$, it controls the toxicity of cadmium ion by lowering its uptake.

Table-1(a): Effect of Cd and solid tea wastage as a biosorbent manure on Shoot and Root Length of *wheat (Triticum aestivum)*

Cd (ppm)	Control	SET -1	SET-2	Cd (ppm)	Control	SET -1	SET-2
		$CdCl_2$	$CdCl_2$ + Tea Waste (5ton/ha)			$CdCl_2$	$CdCl_2$ + Tea Waste (5ton/ha)
Shoot Length (cm)				**Root Length (cm)**			
5		6.44 ±0.86 (-13.6)	6.624 ±0.77 (-11.2)	5		4.252±0.35 (-38.6)	4.34 ±0.47 (-37.37)
10		6.89 ±1.13 (-7.6)	7.83 ±1.14 (+4.95)	10		4.497 ±0.63 (-35.1)	4.74 ±0.75 (-31.6)
15		6.79 ± 0.49 (-8.98)	7.53 ± 0.57 (+0.93)	15		4.293 ±0.31 (-38.05)	4.59 ±0.59 (-33.7)
20		10.66 ±0.24 (+42.8)	11.32 ±1.03 (+51.7)	20		6.886 ±0.96 (-0.63)	13.07 ±0.528 (+88.6)
30		9.87 ±0.85 (+32.3)	10.8 ±0.37 (+44.7)	30		7.16 ±0.89 (+3.31)	8.54 ± 0.68 (+23.2)
40	7.46 ±0.89 (0)	7.91 ±0.22 (+6.03)	8.14 ±2.15 (+9.11)	40	6.93 ±0.20 (0)	4.36 ±1.0 (-37)	6.39 ±1.39 (-7.79)
50		4.696 ±1.25 (-37)	7.07 ±0.39 (-5.22)	50		3.378 ±0.13 (-51.2)	4.04 ±0.21 (-41.7)
60		4.58 ±0.03 (-38.6)	6.56 ±0.05 (-12.06)	60		3.396 ±0.07 (-50.9)	4.58 ±0.34 (-33.9)
100		4.81 ±1.08 (-35.5)	6.51 ±0.63 (-12.7)	100		2.26 ±0.49 (-67.3)	2.36 ±0.08 (-65.9)
250		4.68 ± 1.0 (-37.2)	6.38 ±2.36 (-14.4)	250		1.55 ±0.64 (-85.3)	2.26 ±1.11 (-67.3)
500		1.75 ±0.50 (-76.5)	6.05 ± 4.02 (-18.9)	500		0.42 ±0.07 (-93.3)	2.31 ±1.29 (-66.6)
1000		0 ± 0.0 (-100)	3.28 ±1.12 (-56)	1000		0 ±0.0 (-100)	0.5 ±0.26 (-92.7)

Value in parenthesis indicate percent increase (+) or decrease (-) over control.
Means followed by digits and letter shows significant result at the level of ± Standard Error of Mean.

4.1 *Shoot Length (cm)*

Table-1(a) indicated that, the inhibitory effect of Cd^{2+} on shoot length increased along with raising Cd^{2+} concentration above 40ppm.The elevated Cd^{2+} levels (i.e. 5ppm -1000ppm) significantly reduced wheat shoot length from 7.6% (at 10ppm) to 100% (at 1000ppm), while at 20 - 40ppm, Cd increased shoot length by 42.8%, 32.3% and 6.03% respectively over control. The same phenomena were reported by Kinraide[20]for Al that frequently stimulates plant growth at low concentration. Addition of tea waste influenced the Cd^{2+} toxicity by reducing metal inhibitory effect with respect to shoot length increase. This was due to the absorbent property of tea waste which controls the metal uptake from root, lowered availability of metal ion to the plant tissue, and thus reduced the metal toxicity on growing crop. This increase in growth by tea wastage treatment was in accordance with the finding of Rafia Azmat[21] in *Vigna Radiata* treated with Cr^{2+}metal.

4.2 *Root Length (cm)*

A decrease in root length (Table1-a) showed a fluctuation in inhibitory percentage along the elevated Cd levels, it may be due to the difference in osmotic gradient. There was a minimum reduction i.e 0.63% found with 20ppmCd, raised to 100% at 1000ppm Cd. Table-1a also indicated that, the root length of Wheat plant increased when tea wastage was applied simultaneously with $CdCl_2$, and the increase was highly significant (p~0.05) at all Cd levels, as compared with untreated controls.

4.3 *Plant Fresh Biomass (gm)*

Table-1b indicated that plant fresh mass decreased by Cd^{2+} application from 1.93% at 5ppm Cd to 70.2% at 500ppm Cd. At the level of 50-60ppm Cd, the plant showed slightly positive response toward fresh weight i.e. 1.926% inhibition and 4.203% increase respectively over control.

Table-1(b): Effect of Cd and solid tea wastage as a biosorbent manure on Fresh and Dry Biomass of *wheat (Triticum aestivum)*

Cd (ppm)	Control	SET -1	SET-2
		$CdCl_2$	$CdCl_2$ + Tea Waste (5ton/ha)
Plant Fresh Weight (gm)			
5		0.56±0.144 (-1.926)	0.575±0.004 (+.70)
10		0.48±0.151 (-15.93)	0.6±0.137 (+5.07)
15		0.64±0.016 (+20.84)	0.69±0.032 (+20.84)
20		0.343±0.147 (-39.92)	0.396±0.033 (-30.64)
30		0.383±0.089 (-32.92)	0.426±0.093 (-25.39)
40	0.571 ±0.116 (0)	0.368 ±0.237 (-35.63)	0.458 ±0.197 (-19.87)
50		0.56±0.00 (-1.926)	0.67±0.023 (+17.33)
60		0.595±0.020 (+4.203)	0.715±0.124 (+25.21)
100		0.32±0.0057 (-43.9)	0.361±0.001 (-36.7)
250		0.23±0.0057 (-59.7)	0.29±0.010 (-49.2)
500		0.17 ±0.005 (-70.2)	0.23±0.10 (-59.7±)
1000		0±0.00 (-100)	0.08±0.0057 (-85.9)

Cd (ppm)	Control	SET -1	SET-2
		$CdCl_2$	$CdCl_2$ + Tea Waste (5ton/ha)
Plant Dry Weight (gm)			
5		0.07±0.005 (-16.46)	0.073±0.014 (-12.88)
10		0.06±0.0166 (-28.40)	0.09±0.017 (+7.398)
15		0.093±0.003 (+10.97)	0.096±0.0088 (+14.55)
20		0.077±0.017 (-8.591)	0.097±0.021 (+15.27)
30		0.077±0.0033 (-8.33)	0.1066±0.0088 (+26.90)
40	0.084 ±0.0026 (0)	0.078 ±0.0025 (-7.142)	0.077 ±0.004 (-8.33)
50		0.065±0.0028 (-22.43)	0.08±0.0057 (-4.534)
60		0.075±0.0028 (-10.50)	0.09±0.0057 (+7.398)
100		0.061±0.0052 (-28.7)	0.066±0.0076 (-21.4)
250		0.049±0.0006 (-41.6)	0.053±0.0005 (-36.9)
500		0.032±0.0006 (-61.7)	0.037±0.0005 (-55.9)
1000		0 ±0.00 (-100)	0.019±0.0057 (-77.3)

Value in parenthesis indicate percent increase (+) or decrease (-) over control.
Means followed by digits and letter shows significant result at the level of ± Standard Error of Mean

Tea waste application significantly suppressed metal inhibitory effect on water uptake from external media. This is mainly due to absorptive property of tea waste which lowers the osmotic potential of external solution thus increased water intake from root system. The maximum inhibition of fresh weight (i.e. 70.2%) at 500ppm Cd treated plant was efficiently overcome to 59.7% with tea waste application.

4.4 *Plant Dry Biomass (gm)*

Plant dry weight also followed the same response as the plant length acquired under different Cd levels (Table-1(b)). By increasing the metal concentration, plant dry mass reduced, significantly from 60ppm to onward. This was due to the toxicity of heavy metal above tolerance level. Tea waste extract promoted wheat dry mass accumulation by lowering the metal ion uptake and also by providing some essential elements which promoted the biosynthesis of primary products that enhanced dry weight.

4.5 *Primary and Secondary Metabolites*

Table-2 indicated a significant increase in phenolic content and a gradual decline in the soluble carbohydrate with elevated Cd conc. This was due to heavy metal stress on cell physiological function which reduced the ability and production of carbohydrate by lowering photosynthesis and enhanced the production of antioxidant defense biomolecule (i.e. phenols) (Fig-1) as a conservative strategy against external stress.Table-2 showed a decrease of 35.68% to 72.49% soluble carbohydrate at 50ppm to 150ppmCd and increase of 47.5% total phenol at 100ppmCd over control. This decrease in saccharides content may be due to the increase in diffusion resistance of the stomata to CO_2 and consequently reduced carbon fixation. While, an increase of phenolics, correlated to the increase in activity of enzymes involved in phenolic compounds metabolism suggesting *de novo* synthesis of phenolics under heavy metal stress. This induction of phenolic compound biosynthesis was also observed in wheat in response to Ni^{2+} toxicity[22] and in maize in response to Al^{2+}[23].The addition of tea waste enhanced soluble carbohydrate. This improvement in carbohydrate stuff (Fig-1) was mainly due to the presence of essential elements in tea waste material that facilitated the production and performance of photosynthesis essentials.

Table-2: Effect of Cd Stress and solid tea wastage as a biosorbent manure on Different Biomolecules of *WHEAT (Triticum aestivum)*

TREATMENT		Primary Metabolite	Secondary Metabolite
		Soluble Carbohydrate	Total Phenol
		uM/gmfr.wt	mg/gm Dry wt.
T0	CONTROL	3.469 c (0)	15.45 e (0)
T1	50ppm Cd	2.231 d (-35.68)	14.7 f (-4.85)
T2	50ppm + tea wastage@ 5ton/ha	0.513 g (-295.6)	15.3 e (-0.97)
T3	100ppm Cd	1.379 e (-60.24)	22.8 b (+47.5)
T4	100ppm + tea wastage@ 5ton/ha	7.338 a (+111.5)	25.2 a (+63.1)
T5	150ppm Cd	0.954 f (-72.49)	18.6 d (+20.3)
T6	150ppm + tea wastage@ 5ton/ha	4.535 b (+30.72)	21.3 c (+37.8)

Value in parenthesis indicate percent increase (+) or decrease (-) over control.
Means followed by different letter (a,b,c.) shows significant result at the level of St. deviation.

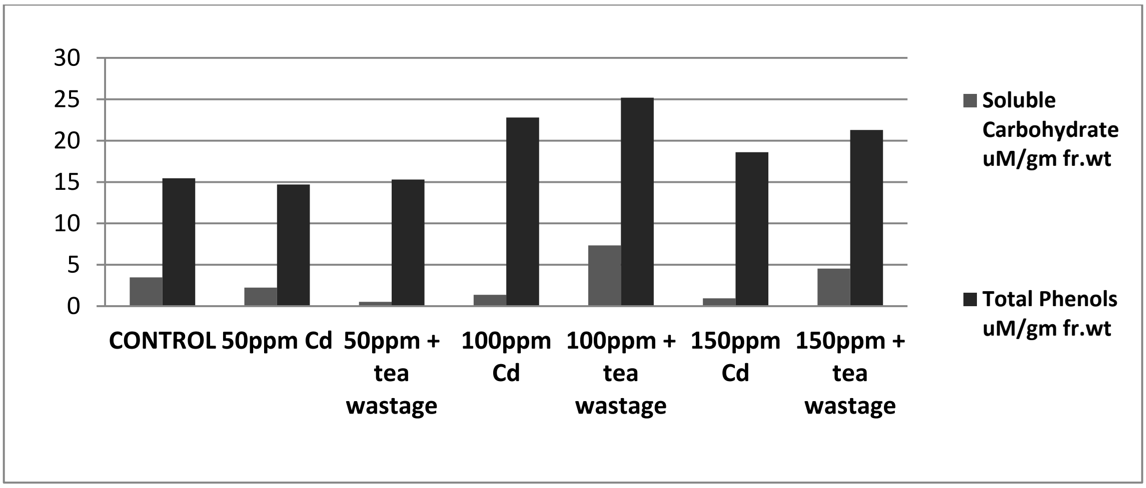

Fig.1: Effect of Cd Stress and Solid tea wastage as biosorbent manure on different Biomolecules of WHEAT (*Triticum aestivum* L.)

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