Effects of macro- and nano- cobalt oxide particles on barley seedlings, and remediation of cobalt chloride toxicity using sodium hypochlorite

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ABSTRACT

This study was undertaken to determine the comparative effects of cobalt (II, III) oxide (Co_3O_4) macro- and nano- particles, and cobaltous chloride hexahydrate ($CoCl_2.6H_2O$) on seed germination, growth and some biochemical parameters of *Hordeum vulgare* L. seedlings. Macro- and nano- Co was added to the sand medium at four levels (50 to 200 mg kg^{-1} sand). Macro- Co was found to increase the growth of both shoots and roots at concentrations up to 200 mg Co kg^{-1} sand. Increase in concentration of nano- Co decreased the root length. Lipid peroxidation was maximum at 200 mg Co kg^{-1} sand for macro- Co in roots. Increase in the lipid peroxidation was found in nano- Co treated roots and shoots. Nano- and macro- Co_3O_4 behaved differently with respect to effects on barley seedlings. The present study also demonstrated the ameliorative effect of NaOCl against $CoCl_2.6H_2O$ toxicity in barley seedlings. NaOCl also decreased the lipid peroxidation induced by $CoCl_2.6H_2O$ and increased chlorophyll content in seedlings.

Keywords: Detoxification; heavy metals; nanotoxicology; sodium hypochlorite**;** *Hordeum vulgare* L.

1. INTRODUCTION

Fast pace of industrialization and irrational use of natural resources has led to metal accumulation in the environment. Metal accumulation in soil is of great concern in agriculture due to its adverse effects on food safety and marketability, plant growth, and soil microflora and fauna [1]. Metal toxicity has high impact on the plants which consequently affect the whole ecosystem due to interdependence of living organisms. Cobalt (Co) is a transition metal with atomic number 27 and atomic weight 58.9 g mol^{-1}. The role of Co in nutrition of leguminous plants is well known, but its importance to the rest of the plant species is still ambiguous [2]. It is an essential element for the synthesis of various enzymes and coenzymes like vitamin B_{12} (cyanocobalamin), which are required for human and animal nutrition. Co is safer for consumption up to 2.5 - 3.0 mg daily, without any adverse health effects [3]. It acts as a coenzyme in a number of cellular processes including the oxidation of fatty acids and the synthesis of DNA. Toxic concentrations of Co inhibit active transport in

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plants. Relatively higher concentrations of Co have toxic effects, including leaf fall, inhibition of greening, discolored veins, premature leaf closure and reduced shoot weight [4].

Two salts of Co are used in industry on a large scale, Co (II, III) oxide, also known as $CoO.Co_2O_3$ or Co_3O_4, macro- and nano- scale particles which are insoluble in water; and cobalt chloride ($CoCl_2.6H_2O$, macroscale particles, water soluble). Nano- Co_3O_4 is a recent discovery and needs to be investigated in detail. $CoCl_2.6H_2O$ is toxic at higher concentrations.

Nanotechnology is the engineered convergence of biology, chemistry and informatics at nanoscale. The products of these exertions are called nanomaterials, consisting of nanoparticles (NPs), having a size smaller than 100 nm in at least one dimension. Among the latest technological innovations, nanotechnology possesses the top position [5]. The properties of nanomaterials raise concern about their potential adverse effects on biological systems at cellular level. Because of their small size, NPs get incursion into the living cell membrane. In contrast to the classical macroscale particles, due to their smaller size and huge surface area, NPs may interact more expeditiously with biological systems. Metal oxide-based NPs are increasingly used in applications such as opacifiers, fillers, catalysts, semiconductors, cosmetics, microelectronics etc. [6]. Therefore, interaction between inorganic nanoparticles and biological systems is one of the most promising areas of research in modern nanoscience and technology.

The present work is aimed at studying the differential effects of macro- and nano- particles of Co_3O_4 and $CoCl_2.6H_2O$ in combination with sodium hypochlorite (NaOCl) on barley seedlings in sand medium. $CoCl_2.6H_2O$ helps in color change in glass industry, organic synthesis and electroplating objects, production of pigments in ceramics and as a mordant in dry cleaners. $CoCl_2.6H_2O$ is a catalyst used for metal surface treatment also. The waste from these industries contains Co more than the prescribed limit. Such industrial effluents when reaching the crop fields cause toxicity to plants [7]. So, to remediate Co rich soil we have tried to use NaOCl for detoxification. NaOCl converts transition metal complexes into their oxides [8]. NaOCl is used in the pesticide and textile industries, and is a disinfectant, cleaner and bleach.

2. MATERIALS AND METHODS

2.1. Study material

Certified and disease-free seeds of barley (*Hordeum vulgare* L.) variety PL-426 were purchased from Punjab Agricultural University, Ludhiana (India). Barley is generally grown as a summer crop in temperate areas and winter crop in tropical areas (including India). It is an important cereal of India, ranking next to wheat, maize and rice in the world.

2.2. Macro- and nano- Co_3O_4 treatments

Salts of Co and other chemicals used in the study were purchased from Sigma-aldrich, Banglore, India; HIMEDIA Laboratory Pvt Ltd; Loba Chemie Pvt Ltd and BTL Research Lab. Suspensions of both macro- and nano- Co_3O_4 were made in distilled water. Different concentrations of both macro- and nano- Co_3O_4 containing 0, 50, 100, 150 and 200 mg Co kg^{-1} sand were prepared respectively.

2.3. $CoCl_2.6H_2O$ and NaOCl treatments

Seeds of barley were grown in sand containing various binary combinations of $CoCl_2.6H_2O$ and NaOCl (Table 1). Growth and biochemical parameters were studied for any modulation in $CoCl_2.6H_2O$ toxicity to seedlings.

2.4. Sand cultures and plant material raising

Seeds of *H. vulgare* were surface sterilized with 0.01% $HgCl_2$ and then washed under running tap water for 10 min. After that, the seeds were soaked in distilled water for 1 h for imbibition. Sand was filtered through sieve of 300 nm size, washed with 0.1 N HCl and thrice with deionised water, and was dried on filter paper in the oven at 80 - 85 ˚C for 3 days. The imbibed seeds were then sown in polypropylene plastic jars of diameter 11 cm containing 0.5 kg sand supplemented with different concentrations of Co. In each jar, 30 seeds of nearly the same size were sown. These sand cultures were maintained at a temperature of 25 ± 0.5˚C, 70 – 80 % relative humidity and 16:8 hour dark : light photoperiod (1700 lux). Then, different plant parts (shoots, roots) were harvested after 7 days of growth for the estimation of root length (RL) and shoot length (SL), fresh weight (fw) and dry weight (dw). Biochemical parameters were studied in terms of oxidative stress caused by metal salts. These included lipid peroxidation and estimation of chlorophyll (chl.) content. Malondialdehyde (MDA) was estimated according to Heath and Packer [9], and chl content was measured by the method described by Arnon [10].

2.5. Statistical analysis

The experimental data were expressed as mean ± SE. One-way and two-way analysis of variance (ANOVA) were done to check the significance of differences within and between treatments, and interactions if any. Significance levels of F- ratios were checked at P = 0.05. Honestly significant differences (HSD) were calculated using Tukey's multiple comparison test at P = 0.05. Difference between any two means in ANOVA, if larger than the HSD value, reveals a statistically significant difference. Linear regression and multiple linear regression with interaction analyses were carried out in MS-Excel using self-coded software. Pearsonian correlation and multiple correlation analyses were done to determine the significance of correlativity among the variables. Unitless beta (β) regression coefficients in multiple regression analysis were calculated in order to measure the relative effects of independent variables (Co, NaOCl and Co×NaOCl interaction) on the dependent variable [11].

3. RESULTS

3.1. Growth characteristics

3.1.1. Co_3O_4 macro- and nano- particles treatment

Seedlings cultured in sand medium containing Co_3O_4 (macro) showed increase in root and shoot length with increase in Co concentration (50, 100, 150 and 200 mg kg^{-1}). Further it was observed that treatment of Co_3O_4 nano- particles significantly increased shoot length but decreased root length (Table 2).

3.1.2. $CoCl_2.6H_2O$ treatments in binary combinations with NaOCl

A significant decrease in shoot, root length and fresh weight (fw) dry weight (dw) of *H. vulgare* was observed upon addition of various concentrations (250, 500, 750 and 1000 mg kg^{-1}) of Co as $CoCl_2.6H_2O$. Further the role of NaOCl as a potent inhibitor of $CoCl_2.6H_2O$ is elucidated in Tables 3, 4 and 5. 750 mg kg^{-1} of NaOCl concentration increased shoot length of seedlings grown in 1000 mg kg^{-1} Co amended sand by 58.57 % and root length by 86.67 %. 500 mg kg^{-1} of NaOCl increased shoot fresh weight of 1000 mg kg^{-1} Co treated seedlings by 91.5 %. Two–way ANOVA variance ratio (F) describes the statistically significant difference among shoot and root lengths on $CoCl_2.6H_2O$ and the NaOCl treatments. Multiple regression models showed that Co has negative effect on shoot and root length, while NaOCl has a positive effect. Interaction between Co and NaOCl was found to be statistically significant. Fresh and dry weight of shoots also showed significant differences (Table 6).

3.2. Lipid peroxidation

Variations in shoot and root MDA content of *H. vulgare* grown in sand media containing Co_3O_4 macro- and nano- particles are presented in Table 7. The MDA content of *H. vulgare* treated with macro- Co_3O_4 was increased significantly for shoots, while a decreasing trend was found in roots. The MDA content for both shoots and roots showed an increasing trend with increase in concentration (50, 100, 150 and 200 mg kg^{-1}) of Co_3O_4 nano- particles in a dose dependent manner. The lowest value for MDA (shoots and roots) was found at concentration of 50 mg Co kg^{-1} sand, while other concentrations showed increased amount of lipid peroxidation. 750 mg kg^{-1} of NaOCl decreased lipid peroxidation in 1000 mg kg^{-1} Co-treated shoots and roots up to 10.65 % and 14.63 % respectively. One-way ANOVA showed significant increase in MDA content in both roots and shoots treated with macro- and nano-Co. Two-way ANOVA revealed that there are significant differences among MDA contents of both shoots and roots in binary treatments (Table 8). The interaction between Co and NaOCl was found to be negative for both shoots and roots (Table 9).

3.3. Chlorophyll estimation

The effects of Co_3O_4 macro- and nano- particles, and binary combinations of $CoCl_2.6H_2O$ with NaOCl on chl content (chl 'a', chl 'b' and total chl) are presented in Tables 10, 11. ANOVA depicted statistical significant differences among different treatments on, chl 'b' and total chl. Multiple regression analysis showed positive effect of NaOCl on chl 'a', which as a result compensated the negative effect of $CoCl_2.6H_2O$. Co and NaOCl significantly increased the chl 'b' content, whereas in the case of total chl, Co showed negative, while NaOCl showed positive β- regression coefficient. It was found that chl 'a', chl 'b' and total chl showed maximum values at 200 mg kg^{-1}. Significant increase was found in the chl 'a', chl 'b' and total chl contents with increase in concentration of Co_3O_4 nano- particles in sand medium. Such results depicted that nano- Co modulated chl synthesis. 500 mg kg^{-1} of NaOCl concentration increased chl 'a', chl 'b' and total chl contents of 1000 mg kg^{-1} Co-treated leaves by 76.06 %, 79.35 % and 77.81 % respectively.

Table 1. $CoCl_2.6H_2O$ treatments (given in numerator) in binary combinations with NaOCl treatments (given in denominator)

NaOCl conc. in sand medium (mg kg^{-1})	$CoCl_2.6H_2O$ conc. (mg kg^{-1}) in sand medium				
	0	**250**	**500**	**750**	**1000**
0	0/0	250/0	500/0	750/0	1000/0
250	0/250	250/250	500/250	750/250	1000/250
500	0/500	250/500	500/500	750/500	1000/500
750	0/750	250/750	500/750	750/750	1000/750
1000	0/1000	250/1000	500/1000	750/1000	1000/1000

Table 2. Effect of Co_3O_4 macro- and nano- particles on root length (RL) and shoot length (SL) (mean ± S.E.) of *H. vulgare* seedlings

Co_3O_4 (mg kg^{-1})	Macro- particles		Nano- particles	
	RL (cm)	SL (cm)	RL (cm)	SL (cm)
0	08.8 ± 0.60	13.8 ± 0.30	12.2 ± 1.40	15.0 ± 1.70
50	09.1 ± 0.80	14.3 ± 0.30	11.6 ± 2.10	16.0 ± 2.40
100	09.9 ± 0.30	14.6 ± 0.10	11.4 ± 1.70	16.4 ± 1.80
150	10.2 ± 0.40	15.0 ± 0.10	10.5 ± 0.67	17.7 ± 1.80
200	11.0 ± 0.10	15.5 ± 0.50	10.1 ± 0.85	18.2 ± 1.90
F- ratio *(*P = 0.5)*	6.84*	12.07*	4.74*	5.17*
HSD	1.53	0.84	2.01	2.72

Table 3. Effect of binary treatments of $CoCl_2.6H_2O$ and NaOCl on shoot length and root length (mean ± S.E.) of *H. vulgare* seedlings

$CoCl_2.6H_2O$ (mg kg^{-1})	NaOCl (mg kg^{-1})									
	0		**250**		**500**		**750**		**1000**	
	SL (cm)	**RL (cm)**	**SL (cm)**	**RL (cm)**	**SL (cm)**	**RL (cm)**	**SL (cm)**	**RL (cm)**	**SL (cm)**	**RL (cm)**
0	11.2 ± 0.50	8.7 ± 0.79	12.2 ± 0.40	8.9 ± 0.39	09.9 ± 0.40	9.0 ± 0.60	10.7 ± 0.60	8.8 ± 0.40	11.0 ± 0.90	09.9 ± 0.52
250	10.9 ± 0.50	8.6 ± 0.43	11.1 ± 0.60	8.3 ± 0.42	10.6 ± 0.70	8.1 ± 0.46	12.2 ± 0.80	9.1 ± 0.45	11.2 ± 0.70	10.7 ± 0.48
500	09.4 ± 1.10	8.4 ± 0.38	12.3 ± 0.60	9.0 ± 0.37	10.1 ± 0.70	8.7 ± 0.26	10.8 ± 0.50	9.6 ± 0.26	10.9 ± 0.80	09.6 ± 0.51
750	07.2 ± 1.10	5.7 ± 0.79	11.3 ± 0.60	9.5 ± 0.47	10.7 ± 0.30	9.6 ± 0.25	09.4 ± 0.90	8.4 ± 0.28	12.1 ± 0.40	09.7 ± 0.42
1000	07.0 ± 1.10	5.3 ± 0.62	09.8 ± 0.30	8.9 ± 0.30	09.6 ± 0.40	8.7 ± 0.66	11.1 ± 0.60	9.8 ± 0.48	11.1 ± 0.40	08.2 ± 0.43

F- ratios for shoots; 4.08* ($CoCl_2.6H_2O$) , 9.21* (NaOCl), 2.39 ($CoCl_2.6H_2O$×NaOCl), **P*= *.05,* HSD= 2.74

F- ratios for roots; 3.47 ($CoCl_2.6H_2O$), 15.97* (NaOCl) , 4.17* ($CoCl_2.6H_2O$×NaOCl), **P*= *.05,* HSD= 1.93

Table 4. Effect of binary treatments of $CoCl_2.6H_2O$ and NaOCl on fresh weight (fw) and dry weight (dw) of shoots (mean ± S.E) of *H. vulgare* seedlings

$CoCl_2.6H_2O$ (mg kg^{-1})	NaOCl (mg kg^{-1})									
	0		**250**		**500**		**750**		**1000**	
	fw (g)	**dw (g)**	**fw (g)**	**dw (g)**	**fw (g)**	**dw (g)**	**fw (g)**	**dw (g)**	**fw (g)**	**dw (g)**
0	1.08 ± 0.05	0.12 ± 0.02	1.73 ± 0.03	0.15 ± 0.007	1.24 ± 0.02	0.10 ± 0.007	1.26 ± 0.01	0.11 ± 0.008	1.43 ± 0.03	0.12 ± 0.004
250	0.84 ± 0.02	0.09 ± 0.004	1.66 ± 0.01	0.15 ± 0.005	1.50 ± 0.01	0.12 ± 0.022	1.32 ± 0.02	0.11 ± 0.009	1.55 ± 0.06	0.14 ± 0.006
500	0.81 ± 0.01	0.08 ± 0.003	0.85 ± 0.01	0.08 ± 0.003	0.72 ± 0.03	0.07 ± 0.005	0.67 ± 0.06	0.05 ± 0.009	1.26 ± 0.02	0.11 ± 0.008
750	0.75 ± 0.03	0.08 ± 0.004	0.87 ± 0.01	0.07 ± 0.002	1.14 ± 0.01	0.11 ± 0.006	1.05 ± 0.01	0.09 ± 0.003	1.16 ± 0.02	0.11 ± 0.006
1000	0.71 ± 0.01	0.07 ± 0.003	1.11 ± 0.01	0.10 ± 0.008	1.36 ± 0.03	0.14 ± 0.007	1.15 ± 0.05	0.11 ± 0.007	1.72 ± 0.02	0.16 ± 0.010

F- ratios for shoots (fw) ; 990.59* ($CoCl_2.6H_2O$), 915.10*(NaOCl), 153.83* ($CoCl_2.6H_2O$×NaOCl), *P= .05,* HSD= 0.07

F- ratios for shoots (dw) ; 81.48* ($CoCl_2.6H_2O$), 48.05* (NaOCl), 16.36* ($CoCl_2.6H_2O$×NaOCl), *P= .05,* HSD= 0.02

Table 5. Effect of binary treatments of $CoCl_2.6H_2O$ and NaOCl on fresh weight (fw) and dry weight (dw) of roots (mean ± S.E) of *H. vulgare* seedlings

$CoCl_2.6H_2O$ ($mg\ kg^{-1}$)	**NaOCl ($mg\ kg^{-1}$)**									
	0		**250**		**500**		**750**		**1000**	
	fw (g)	**dw (g)**	**fw (g)**	**dw (g)**	**fw (g)**	**dw (g)**	**fw (g)**	**dw (g)**	**fw (g)**	**dw (g)**
0	1.21 ± 0.07	0.07 ± 0.003	1.68 ± 0.05	0.15 ± 0.005	1 .02 ± 0.01	0.09 ± 0.005	1.02 ± 0.01	0.08 ± 0.007	1. 13 ± 0.11	0.08 ± 0.004
250	1.04 ± 0.06	0.07 ± 0.007	1.35 ± 0.13	0.14 ± 0.007	1 .12 ± 0.04	0.10 ± 0.001	0.91 ± 0.01	0.08 ± 0.002	1. 34 ± 0.06	0.09 ± 0.003
500	0.94 ± 0.03	0.06 ± 0.008	0.67 ± 0.04	0.05 ± 0.006	0 .87 ± 0.04	0.05 ± 0.009	0.54 ± 0.02	0.09 ± 0.004	1. 2 ± 0.03	0.11 ± 0.004
750	0.85 ± 0.05	0.06 ± 0.007	0.73 ± 0.04	0.06 ± 0.009	0 .95 ± 0.04	0.07 ± 0.005	0.83 ± 0.03	0.09 ± 0.004	1. 01± 0.01	0.11 ± 0.006
1000	0.74 ± 0.04	0.05 ± 0.007	0.96 ± 0.01	0.13 ± 0.007	1 .03 ± 0.02	0.09 ± 0.011	1.02 ± 0.01	0.09 ± 0.007	1. 31 ± 0.07	0.11 ± 0.003

F- ratios for roots (fw) ; 162.88* ($CoCl_2.6H_2O$), 97.04* (NaOCl), 44.21* ($CoCl_2.6H_2O$×NaOCl) , **P= .05,* HSD= 0.13

F- ratios for roots (dw) ; 71.07* ($CoCl_2.6H_2O$) , 31.17* (NaOCl), 64.99* ($CoCl_2.6H_2O$×NaOCl) , **P= .05,* HSD= 0.02

Table 6. Multiple regression models for shoot length (SL) and root length (RL), fresh weight (fw) and dry weight (dw) of *H. vulgare* seedlings in binary combination of $CoCl_2.6H_2O$ (mg kg^{-1}) and NaOCl (mg kg^{-1})

Dependent variable (Y)	Multiple regression equation	r	β-regression coefficients		
			Co	NaOCl	Co×NaOCl
SL (cm)	Y= 11.69 – 0.0038 Co – 0.0008 NaOCl + 5×10^{-6} Co×NaOCl	0.720*	- 1.02	-0.22	0.99
RL (cm)	Y= 8.66 – 0.0017 Co + 0.0011 NaOCl + 2×10^{-6} Co×NaOCl	0.673*	- 0.53	0.33	0.41
Shoot fw (g)	Y= 1.23 – 0.0005 Co + 0.0001 NaOCl + 6×10^{-7} Co×NaOCl	0.58*	- 0.61	0.13	0.50
Shoot dw (g)	Y= 8.66 – 0.0017 Co + 0.0011 NaOCl + 2×10^{-6} Co×NaOCl	0.673*	- 0.53	0.33	0.41
Root fw (g)	Y= 1.27 – 0.0002 Co – 0.0006 NaOCl + 7×10^{-7} Co×NaOCl	0.56*	-0.35	-0.90	0.79
Root dw (g)	Y= 0.095 – 0.00 Co – 0.00 NaOCl + 6×10^{-8} Co×NaOCl	0.47#	- 0.12	-0.50	0.65

**P*= .05, #*P*= .10

Table 7. Lipid peroxidation (µ mole MDA; mean ± S.E) of *H. vulgare* seedlings after treatment with Co_3O_4 macro- and nano-particles

Co_3O_4 (mg kg^{-1})	**Macro- particles**		**Nano- particles**	
	MDA shoots	**MDA roots**	**MDA shoots**	**MDA roots**
0	2.72 ± 0.04	1.98 ± 0.04	1.71 ± 0.12	1.18 ± 0.02
50	2.43 ± 0.18	1.74 ± 0.01	1.26 ± 0.04	1.26 ± 0.04
100	2.24 ± 0.18	1.54 ± 0.01	1.65 ± 0.12	1.28 ± 0.01
150	2.48 ± 0.03	1.50 ± 0.03	1.78 ± 0.06	1.64 ± 0.11
200	2.99 ± 0.03	0.91 ± 0.05	1.97 ± 0.06	1.71 ± 0.12
F-ratio *(*P= .05)*	17.77*	466.81*	63.05*	31.99*
HSD	0.31	0.086	0.15	0.19

Table 8. Lipid peroxidation (μ mole MDA ; mean ± S.E) of *H. vulgare* seedlings after binary treatments with $CoCl_2.6H_2O$ and NaOCl

$CoCl_2.6H_2O$ (mg kg^{-1})	NaOCl (mg kg^{-1})									
	0		250		500		750		1000	
	MDA shoots	MDA roots	MDA shoots	MDA roots	MDA shoots	MDA roots	MDA shoots	MDA roots	MDA shoots	MDA roots
0	2.76 ± 0.03	0.39 ± 0.05	2.96 ± 0.032	0.45 ± 0.05	2. 54 ± 0.012	0.42 ±0.08	2.15 ± 0.006	0.37 ±0.07	3.17 ± 0.01	0.52 ± 0.12
250	2.87 ± 0.02	0.50 ± 0.06	2.93 ± 0.006	0.68 ± 0.15	2. 65 ± 0.006	0.48 ±0.03	2.28 ± 0.006	0.44 ±0.06	2.99 ± 0.01	0.56 ± 0.06
500	2.96 ± 0.03	0.59 ± 0.06	3.11 ± 0.006	0.52 ± 0.03	2. 36 ± 0.006	0.63 ±0.07	3.21 ± 0.05	0.40 ±0.06	2.89 ± 0.01	0.53 ± 0.03
750	3.12 ± 0.08	0.69 ± 0.11	3.49 ± 0.005	0.51 ± 0.16	2. 88 ± 0.006	0.53 ±0.07	3.03 ± 0.02	0.49 ±0.02	3.08 ± 0.03	0.63 ±0.07
1000	3.66 ± 0.04	0.82 ± 0.08	2.96 ± 0.017	0.39 ± 0.04	2. 09 ± 0.006	0.54 ±0.01	3.27 ± 0.01	0.70 ±0.08	3.2 ± 0.006	0.44 ± 0.07

F- ratios for MDA (Shoots) ; 399.79* ($CoCl_2.6H_2O$), 850.19* (NaOCl), 262.63* ($CoCl_2.6H_2O$×NaOCl) , **P*= *.05,* HSD= 0.09

F- ratios for MDA (Roots) ; 8.37* ($CoCl_2.6H_2O$), 4.79* (NaOCl) , 6.22* ($CoCl_2.6H_2O$×NaOCl) , **P*= *.05,* HSD= 0.21

Table 9. Multiple regression models for lipid peroxidation (μ mole MDA g^{-1} tissue) in shoots and roots, and chl content (mg g^{-1} fw) of *H. vulgare* in binary combinations of $CoCl_2.6H_2O$ and NaOCl

Dependent variable (Y)	Multiple regression equation	r	β-regression coefficients		
			Co	NaOCl	Co×NaOCl
MDA shoot	Y= 2.71 – 0.0005 Co – 2×10^{-5} NaOCl – 2×10^{-7} Co×NaOCl	0.40#	0.48	-0.016	-0.16
MDA root	Y= 0.44 – 0.0002 Co + 4×10^{-5} NaOCl – 2×10^{-7} Co×NaOCl	0.52*	0.76	0.14	0.53
Chl ‘a’	Y= 5.35 – 0.0013 Co – 0.0009 NaOCl + 2×10^{-6} Co×NaOCl	0.27	-0.44	-0.28	0.56
Chl ‘b’	Y= 2.48 – 0.0008 Co – 0.0002 NaOCl + 2×10^{-6} Co×NaOCl	0.37#	-0.35	-0.11	0.58
Total Chl	Y= 7.83 – 0.0021 Co – 0.0011 NaOCl + 4×10^{-6} Co×NaOCl	0.31	-0.40	-0.21	0.59

**P*= .05, #*P*= .10*

Table 10. Chlorophyll content (mean ± S.E) of *H. vulgare* after treatment with Co_3O_4 macro- and nano- particles

Co_3O_4 (mg kg^{-1})	**Chl Content**					
	Chl 'a' (mg g^{-1} fw)		**Chl 'b' (mg g^{-1} fw)**		**Total Chl (mg g^{-1} fw)**	
	Macro- particles	**Nano- particles**	**Macro- particles**	**Nano- particles**	**Macro- particles**	**Nano- particles**
0	0.60 ± 0.004	0.61 ± 0.01	0.13 ± 0.004	0.12 ± 0.006	0.73 ± 0.003	0.73 ± 0.02
50	0.37 ± 0.02	0.49 ±0.02	0.19 ± 0.003	0.18 ± 0.003	0.54 ± 0.003	0.68 ± 0.03
100	0.45 ± 0.04	0.52 ±0.021	0.21 ± 0.004	0.19 ± 0.003	0.65 ± 0.005	0.73 ± 0.07
150	0.52 ± 0.01	0.54 ± 0.05	0.23 ± 0.03	0.26 ± 0.03	0.76 ± 0.01	0.79 ± 0.02
200	0.62 ± 0.003	0.68 ±0.03	0.28 ± 0.04	0.27 ± 0.02	0.91 ± 0.003	0.94 ± 0.02
F- ratios *(*P= .05)*	78.25*	22.72*	21.72*	44.11*	1805.92*	26.54*
HSD	0.45	0.57	0.44	0.33	0.12	0.75

Table 11. Chlorophyll content (mg g^{-1} fw) of *H. vulgare* seedlings after binary treatments with $CoCl_2.6H_2O$ and NaOCl

$CoCl_2.6H_2O$ (mg kg^{-1})	NaOCl (mg kg^{-1})														
	0			250			500			750			1000		
	Chl 'a'	**Chl 'b'**	**Total Chl**	**Chl 'a'**	**Chl 'b'**	**Total Chl**	**Chl 'a'**	**Chl 'b'**	**Total Chl**	**Chl 'a'**	**Chl 'b'**	**Total Chl**	**Chl 'a'**	**Chl 'b'**	**Total Chl**
0	0.52 ± 0.06	0.25 ± 0.05	0.77 ± 0.05	0.49 ± 0.07	0.25 ± 0.07	0.75 ± 0.03	0.41 ± 0.004	0.18 ± 0.02	0.59 ± 0.02	0.42 ± 0.004	0.21 ± 0.02	0.62 ± 0.02	0.40 ± 0.09	0.18 ± 0.03	0.58 ± 0.03
250	0.51 ± 0.04	0.24 ± 0.04	0.75 ± 0.05	0.58 ± 0.02	0.26 ± 0.04	0.85 ± 0.07	0.40 ± 0.004	0.19 ± 0.05	0.59 ± 0.01	0.55 ± 0.04	0.27 ± 0.04	0.82 ± 0.03	0.47 ± 0.05	0.22 ± 0.07	0.68 ± 0.01
500	0.44 ± 0.02	0.21 ± 0.07	0.66 ± 0.05	0.41 ± 0.004	0.20 ±0.002	0.62 ± 0.03	0.72 ± 0.06	0.34 ± 0.03	1.06 ± 0.08	0.62 ± 0.07	0.28 ± 0.03	0.90 ± 0.05	0.352 ± 0.041	0.18 ± 0.03	0.53 ± 0.03
750	0.43 ± 0.03	0.20 ± 0.02	0.64 ± 0.05	0.36 ± 0.041	0.19 ± 0.010	0.55 ± 0.06	0.48 ± 0.02	0.25 ± 0.05	0.73 ± 0.04	0.59 ± 0.08	0.29 ± 0.02	0.88 ± 0.07	0.16 ± 0.04	0.06 ± 0.01	0.22 ± 0.02
1000	0.38 ± 0.01	0.18 ± 0.04	0.56 ± 0.05	0.45 ± 0.01	0.23 ± 0.01	0.68 ± 0.03	0.66 ± 0.03	0.33 ± 0.01	0.99 ± 0.06	0.61 ± 0.02	0.53 ± 0.01	1.14 ± 0.03	0.55 ± 0.04	0.25 ± 0.05	0.79 ± 0.1

F- ratios for Chl 'a', for binary treatments; 13.88* ($CoCl_2.6H_2O$), 25.84* (NaOCl) , 13.24* ($CoCl_2.6H_2O$×NaOCl) , *P= .05,* HSD= 1.41

F- ratios for Chl 'b' for binary treatments; 20.82* ($CoCl_2.6H_2O$) , 32.89* (NaOCl), 11.52* ($CoCl_2.6H_2O$×NaOCl) , *P= .05,* HSD= 0.94

F- ratios for Total Chl for binary treatments; 56.05* ($CoCl_2.6H_2O$),106.98* (NaOCl), 42.43* ($CoCl_2.6H_2O$×NaOCl) , *P= .05,* HSD= 1.20

4. DISCUSSION

Heavy metals may cause major occupational and environmental hazards due to their non-biodegradable nature and long biological half life period [12]. Exposure to heavy metals is mainly due to the anthropogenic actions such as use of fertilizers, agrochemical compounds, sewage sludge and other activities like mining [13]. Such activities result in the transportation of metal ions via air and water, which ultimately bind to soil and sediments. Co is a relatively rare magnetic element with properties similar to those of iron and nickel, and occurs in nature primarily as arsenides, oxides and sulphides. Most of the production of Co involves the metallic form used in the formation of Co superalloys [14]. The distribution of Co in plants is entirely species specific.

A significant increase in both root and shoot length was observed in 7 days old seedlings treated with Co_3O_4 macro-particles, while treatment of Co_3O_4 nano- particles increased only shoot length. These observations are in accordance with earlier studies [15] where cobalt is said to increase the seedling growth by alleviating the senescence of aged tissue by inhibiting the activity of 1-aminocyclopropane-1-carboxylate (ACC) oxidase, and reducing ethylene production. $CoCl_2.6H_2O$ was found to be toxic at higher concentrations as was observed from decreased root and shoot length. NaOCl decreased $CoCl_2.6H_2O$ induced decrease in both root and shoot length. NaOCl is known to transform Co into its oxide form either through exclusion, inclusion (i.e. sequestration and compartmentalization of metal ions in organelles) or chelation binding [16]. The reaction of $CoCl_2.6H_2O$ with NaOCl is given below:

$$3CoCl_2.6H_2O + 6NaOCl \xrightarrow{\text{Aqueous}} Co_3O_4\ (ppt) + 6NaCl + 3Cl_2 + 4O_2$$

The reason for such an observation might be attributed to the fact that NaOCl oxidises the more toxic $CoCl_2.6H_2O$ to less toxic Co_3O_4 [8]. At treatments where NaOCl was absent altogether, metal-caused toxicity resulted in reduction of shoot length. Lowest shoot length was observed at concentrations where Co is in maximum and NaOCl is in minimum amounts. The amount of NaOCl required for counteracting toxicity caused by Co is more in the case of roots as compared to shoots. This may be attributed to the fact that roots are accumulative organs of heavy metals [17].

Lipid peroxidation was found to be maximum for roots at a concentration of 200 mg kg^{-1} of Co_3O_4. The reason for such a trend can be attributed to increased production of ROS which induce membrane destabilization resulting in the formation of peroxides, as was reported by Mead et al. [18]. On the other hand, Co_3O_4 inhibited lipid peroxidation by decreasing the MDA content in roots and the differences obtained were statistically significant.

A significant reduction in chl content (chl ‘a’, chl ‘b’ and total chl) induced by $CoCl_2.6H_2O$ as compared to the untreated control might be due to overproduction of reactive oxygen species, which in turn could have damaged chloroplast membrane [19] as was observed on the effect of Co on *Cajanus cajan* Mill. Application of NaOCl in combination with $CoCl_2.6H_2O$ increased levels of Chl ‘a’, Chl ‘b’ and total Chl. Protection extended by NaOCl was evident from the fact that it significantly reduced ROS production as was observed in lipid peroxidation studies.

5. CONCLUSION

Our results showed that Co_3O_4, both, nano- as well as macro- particles showed differential toxic effects on *H. vulgare* seedlings. Furthermore, the application of NaOCl significantly reduced the toxicity caused by $CoCl_2.6H_2O$ in *H. vulgare* seedlings. Improved cobalt stress mitigation by NaOCl involves biochemical ramifications. Thus, the present study presents NaOCl as effective candidate in ameliorating $CoCl_2.6H_2O$ toxicity.

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