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Phytotoxic effect of leachates of industrial solid waste on the growth of *Pisum sativum*

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Abstract

The present investigation deals with the study on the phytotoxicity of leachates obtained from Eveready Industries India Ltd., Flashlight Lucknow. Analysis of heavy metals in leachates and effluent showed that leachates contained heavy metals in the following order Ni>Zn>Cr>Cu, whereas effluent mainly contained Hg in high (0.22 $\mu g\ ml^{-1}$) concentration. Phytotoxicity of leachates on different parameters of *P. sativum* was studied in pot experiment. The results revealed that leachate induced concentration dependent decline in the germination percentageand protein content. However, lower concentration of leachate (5-20%) showed stimulatory effect on the chlorophyll and carotenoid content. A continuous dumping of such solid waste in the vicinity of populated area will ultimately cause surface and ground water pollution due to leaching of the waste.

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Heavy metal, Leachate, Phytotoxicity, *Pisum sativum*

Introduction

Solid waste can be a major source of contamination to groundwater and surface water (Zaporozec, 2006; Longe and Enekwechi, 2007). Contamination of environment by landfill leachates mainly occurs due to uncontrolled over-flow, rainfall run-off, or infiltration. The water run-offs and leachates from these wastes is expected to be toxic to human health or plants, and has deleterious effects on the physiology of living systems (Chandra *et al.,* 2004; Alimba *et al*., 2006; Bortollo *et al*.,2009). Previous studies have reported that metal intake by plants increases with increasing heavy metal concentrations in the soil solutions (Huang *et al.,* 2007). Therefore, animal and plant bioassays have been used to detect the toxicity of such leachates (Isidori *et al*., 2003; Blinova, 2004; Pratt *et al*.,2004). US Environmental Protection Agency (US EPA, 2006) has also suggested the use of test organisms to establish the toxicity of the environmental pollutants. Many plant species are used for testing of eco toxicity in terms of seed germination and root elongation (Chaignon and Hinsinger, 2003; Srivastava *et al*., 2005). Several studies have been conducted to evaluate the effects of different metals on plants (Thompson *et al.,* 1997). The legumes are shown to be more sensitive to metals (Marchiol *et al*., 1999; Rana and Masood, 2002). Various sudies show that legume crops are highly responsive to leachates in terms of growth and seed germination (Kumar *et al.*, 2012).

Eveready Industries India Ltd., a flashlight electro plating plant, located at Lucknow,is known to release both solid and liquid wastes rich in toxic metals. In view of the above, the present study aimed to evaluate the response of *Pisum sativum* to different concentrations of leachates in terms of percent phytotoxicity, germination index, metal tolerance index, total chlorophyll and protein content, respectively.

Materials and Methods

Study area : The effluent was collected in a translucent bottle from an outlet pipe situated half kilometers away from industrial area of Eveready Industries India Ltd. The bluish-green solid-waste generated by this industry was collected from the dumping pits of the industry and was used to prepare the leachate solution as per EPA Test Method 1311 (1992). The leachates and industrial effluent was analyzed for heavy metals by Inductive Couple Plasma Emission Spectro photometer (Thermo Electron, U.S.A. model IRIS INTERPAID II XDLμ). Soil samples collected from the garden of Babasaheb Bhimrao Ambedkar University, Lucknow, served as control.

Seed germination: Seeds of *Pisumsativum* (AP3 variety) were taken to study the effects of leachates on seed germination and growth of *Pisum sativum*. Seeds were sterilized with 0.1% mercuric chloride solution to avoid surface fungal/bacterial contamination (Young, 1926). The effects of varying concentrations of leachates were studied on the germination of pea seeds. The seeds were pre-soaked in distilled water for overnight before placing in sterilized Petri dishes of uniform size lined with double layer of Whatman No. 42 filter papers. The different concentrations (0, 5, 10, 15, 20 and 25% v/v) of leachates were prepared by using 10% Hoagland solution. The Petri dish containing Hoagland solution without leachate served as control. The test was performed in triplicate for each concentration. Nine seeds were taken in each petri dish and covered petridishes were placed at room temperature for one week. The germination rate and length of radicle was measured after a week. Emergence of radicles of more than 1.0 mm size was taken as a criterion of germination.

Assesment of phytotoxicity : Phytotoxicity was deduced from the effect of leachates on radicle growth using the formula given by Chou *et al. (*1976) and modified by Ray and Banerjee (1981). Metal tolerance index was calculated from the radicle length using the formula given by Turner and Marshal (1972). Germination index was calculated according to the equation given by IRSA (1983).

Pot experiment: Nine seeds were sown in pots filled with soil and sand (3:1, w/w) in triplicates. The seeds were irrigated with different concentrations (5, 10, 20, 40, 70 and 100 % v/v) of leachates on every 3rd day. After 4 weeks of leachate treatment, the plants were harvested and physical parameter like root length, height of plant was measured. The pot irrigated with Hoagland solution without leachate served as control.

Biochemical analysis : After 4 week of leachate treatment, the chlorophyll and carotenoid contents in the leaves were estimated following the method of Machalachan and Zalik (1963) while the protein was estimated by the method of Lowry *et al.* (1951).

Statistical analysis : Data in triplicate (n=3) were analyzed by *t*-test and one way analysis of variance (ANOVA), using Duncan Multiple Range Tests (DMRT) to determine the significant difference among treatments at $p<0.05$and 0.01.

Results and Discussion

The physico-chemical characteristics of soil and solid waste are given in Table 1. Except for bulk density, water holding capacity and redox potential, all the parameters were found significantly higher ($p<0.05$ and 0.01) in the solid waste than control soil.

The metal contents in the effluent discharge from the Eveready Industry was analyzed along with the leachate prepared from its solid waste (Table 2). It was observed that effluent contained Hg above the permissible limit as per CPCB (1987). Whereas other metals were present within the prescribed limit. On the other hand, Cr, Cu, Ni, Hg and Zn were present in higher quantities in the leachates. The industrial solid waste of the Flashlight Industry is being dumped directly in the soil, contains higher quantity of metals than that is present in the effluent. The garden soil contained low concentration of heavy metals decreasing order of Zn>Cu>Pb>Ni>Cr>Hg except for Fe and Mn in the (Table 2) and leachates. Thus, a study on the direct effect of the solid waste leachates on the seed germination and growth of *Pisum sativum* seems quite relevant to assess the phytotoxic nature of the solid waste.

Table 1 : Physico-chemical characteristics of solid waste

Parameter	Control soil	Solid waste
pH	7.03±0.10	7.84±0.10^{ns}
Bulk density (mg g^{-3})	1.36±0.09	0.76±0.02*
Cation exchange capacity (Cmol (P+) kg^{-1})	28.43±2.95	172.47±9.98**
Organic carbon (%)	0.279±0.03	4.18±0.09**
Organic matter (%)	0.62±0.02	9.14±0.23**
N-NO_3(mg kg^{-1})	6.18±0.39	31.75±1.50**
N-NH_4 (mg kg^{-1})	2.56±0.17	9.54±1.28**
Available phosphorus (μg g^{-1})	3.26±0.27	16.64±1.62**
Water holding capacity (%)	61.13±7.89	59.23±4.91^{ns}
Redox potential (mV)	226.50±18.14	82.07±15.87**

All the values are means of three replicates ± S.E, ns = not significant, significant *=$p < 0.05$and **= $p<0.01$

Effect of different concentrations of leachate (5-25%) on seed germination is given in Table 3. Results showed a concentration dependent decrease in the germination percentage and radicle growth of pea seeds. Only 7.4% germination was observed at 25% leachate concentration. Beyond this concentration, the percent germination was almost negligible. A concentration dependent decrease in metal tolerance index and germination index for *Pisum sativum* clearly exhibited toxic nature of the leachate. Claire *et al.* (1991) reported toxic effect of Ni and other heavy metals on cabbage, lettuce, millet, radish, turnip, and wheat.The decrease in metal tolerance index and increase in phytotoxicity with increase in heavy metal including Ni, Cu and Zn concentrations in the rhizosphere has also been reported in *Cicer arientinum* and *Phaseolus mungo* (Kumar *et al.,* 2009), *Brassica juncea* (Bauddh *et al*., 2009; 2011) and *Vigna mungo* (Solanki *et al.,* 2011). Whereas, studies related to fly ash leachates have shown improvement in the crop productivity (Khan and Khan, 1996). There is a possibility that distillery and industrial sludge leachates at lower concentrations cater to the trace element requirement of the plant and thereby, improve the crop productivity (Mishra and Pandey,2002). On the contrary, our results demonstrate that solid waste of the Eveready Industry was highly toxic. Leachates of the solid waste of this industry may not improve the soil fertility as it contained toxic metals such as Hg, Cr and Pb (Melekhova *et al*., 2006).

The plants irrigated with different concentrations of leachates decreased the root and shoot length (Table 4). Only 43% seed germination was observed at 100% leachate concentration. This reduction in germination and growth of

Table 2 : Metal concentrations in discharged effluent, leachates and solid waste of Industry

Metals	Control soil (mg g^{-1} d. wt.)	Solid waste (mg g^{-1} d. wt.)	Leachates (mg ml^{-1})	Industrial effluent (mg ml^{-1})	Permissible limit* of metals (mg ml^{-1}) (CPCB, 1987)
Ni	1.81±0.44	1323.4 ± 2.56	66.17 ± 1.27	0.07 ± 0.01	3.0
Zn	22.26±1.55	594.20 ± 1.56	29.71 ± 1.02	0.02 ± 0.01	5.0
Cr	0.22±1.26	368.0 ± 1.32	18.40 ± 0.98	1.49 ± 0.02	2.0
Cu	5.95±0.78	304.80 ± 1.30	15.24 ± 0.80	0.10 ± 0.01	3.0
Mn	68.21±8.19	15.46 ± 0.79	0.77 ± 0.04	0.13 ± 0.01	2.0
Hg	—	0.840 ± 0.04	0.42 ± 0.02	0.22 ± 0.03	0.01
Pb	2.21±0.30	38.2 ± 1.34	0.21 ± 0.01	0.08 ± 0.01	0.1
Fe	332.1±1.5	30.2 ± 2.7	0.15 ± 0.01	0.34 ± 0.02	3.0

Values are mean of three replicates ±S.E

Table 3 : Effect of different concentration of leachate on seed germination of *Pisum sativum* in Hoagland solution

Concentration %	Radicle length (cm)	Germination (%)	Phytotoxicity (%)	MTI (%)	GI (%)
0	2.70 ± 0.30^{a}	100±0.00^{a}			
5	2.30 ± 0.10^{a}	33.3±0.27^{b}	14.81±0.04^{d}	85.18^{a}	28.37±1.2^{a}
10	1.87 ± 0.20^{b}	18.81±0.08^{c}	30.74 ±1.25^{c}	69.25^{b}	10.26±0.89^{b}
15	1.10 ± 0.02^{c}	13.1±0.064^{d}	59.25±4.52^{b}	40.74^{c}	4.52±0.12^{c}
20	0.40 ± 0.02^{d}	11.1±0.12^{d}	85.18±4.87^{ab}	14.81^{d}	1.64±0.08^{d}
25	0.15 ± 0.01^{de}	7.40±0.03^{e}	94.44±5.26^{a}	5.55^{e}	0.41±0.01^{e}

Values are mean of three replicates ±S.E. Values followed by different letters are significantly different $p<0.05$

Table 4 : Effect of leachate on the morphological parameters of *Pisum sativum* in pot experiment

Concentration %	Root length (cm)	Shoot length (cm)	Percent germination	Percent phyto-toxicity	Metal tolerance index	Percent germination index
0	7.5 ± 1.04^{a}	9.56 ± 1.0^{a}	100.0±0.00^{a}	—	—	—
5	7.2 ± 0.43^{a}	7.05 ±0.76^{b}	77.77±0.24^{b}	4.0±0.25^{d}	96.00±4.25^{a}	74.59±2.25^{a}
10	5.2 ± 0.43^{b}	6.62 ± 0.52^{bc}	67.45±4.13^{c}	30.6±1.35^{cd}	69.33±3.26^{b}	46.76±4.15^{b}
20	5.0 ± 0.31^{b}	6.01 ± 0.62^{bc}	61.77±2.14^{d}	33.3±2.24^{cd}	66.66±2.59^{b}	41.18±4.17^{b}
40	4.5 ± 0.30^{c}	5.49 ± 0.41^{c}	57.43±4.55^{d}	40.0±1.54^{c}	60.0±1.24^{bc}	34.45±2.01^{c}
70	3.8 ± 0.22^{cd}	4.86 ± 0.12^{cd}	49.75±2.78^{e}	49.3±3.58^{b}	50.66±2.35^{c}	25.20±1.05^{d}
100	2.6 ± 0.24^{cd}	3.58± 0.20^{d}	43.50±3.54^{f}	65.3±4.56^{a}	34.66±2.11^{d}	15.08±0.89^{e}

Values are mean of three replicates ±S.E . Values followed by different letters are significantly different $p< 0.05$

pea plant may be due to the adverse effect of heavy metals present in the leachate. Heavy metal toxicity to plants is manifested in terms of stunted growth, leaf chlorosis and alteration in the activity of key enzymes of various metabolic pathways (Bharti and Singh, 1994; DiToppi and Gabbrielli, 1999; Chaudhary and Singh, 2000; Sharma *et al*., 2010). A comparison of the results on seed germination obtained with Petri dish and pot experiment revealed a reduced toxic effect of leachate on the seed germination in pot experiment. This differential response of pea seeds may be due to difference in the mobilization of metals in the surrounding matrix.

The effect of leachate treatment after 4 weeks of germination on pigment content of pea plant and protein is shown in Fig. 1 (A-C) . The result showed an increase in the total chlorophyll and carotenoid contents upto 20% leachate concentration. Beyond this concentration, the level of pigments gradually decreased. The increase in the level of chlorophyll and carotenoid pigments at extremely low concentrations of leachate (20 %) may be due to positive impact of other micronutrients on the pea plant. There are various reports about heavy metals (Cu, Ni, Cr and Cd) induced increase in the chlorophyll content (Pandey and Sharma, 2002; Singh *et al*., 2004; Aravind and Prasad, 2005; Rahman*et al.,* 2005; Bauddh and Singh, 2011) and photosynthetic electron transport (Scholnick and Keren, 2006). Whereas, the protein content in the leaves decreased with increase in leachate concentration. These results suggested that unlike pigment, the protein synthesis in pea plant was relatively more sensitive to metal leachates. Similar decrease in protein content with increase in Ni, Cu, Cd and Zn level has been reported in *H. annuus* and *B. juncea* (Finkriye and Kirbag, 2007; Bauddh and Singh, 2011). However, Kumar *et al. (*2002) and Tripathi *et al.* (2004) reported increase in protein content which might be considered as defense strategy of plants to cope up with fly ash toxicity.

From the foregoing study, it is concluded that the solid waste generated by the Eveready Industry is unfit for its disposal in the agricultural soil due to its toxicity to the plants. A positive correlation between the soil matrix and leachate toxicity needs to be investigated.

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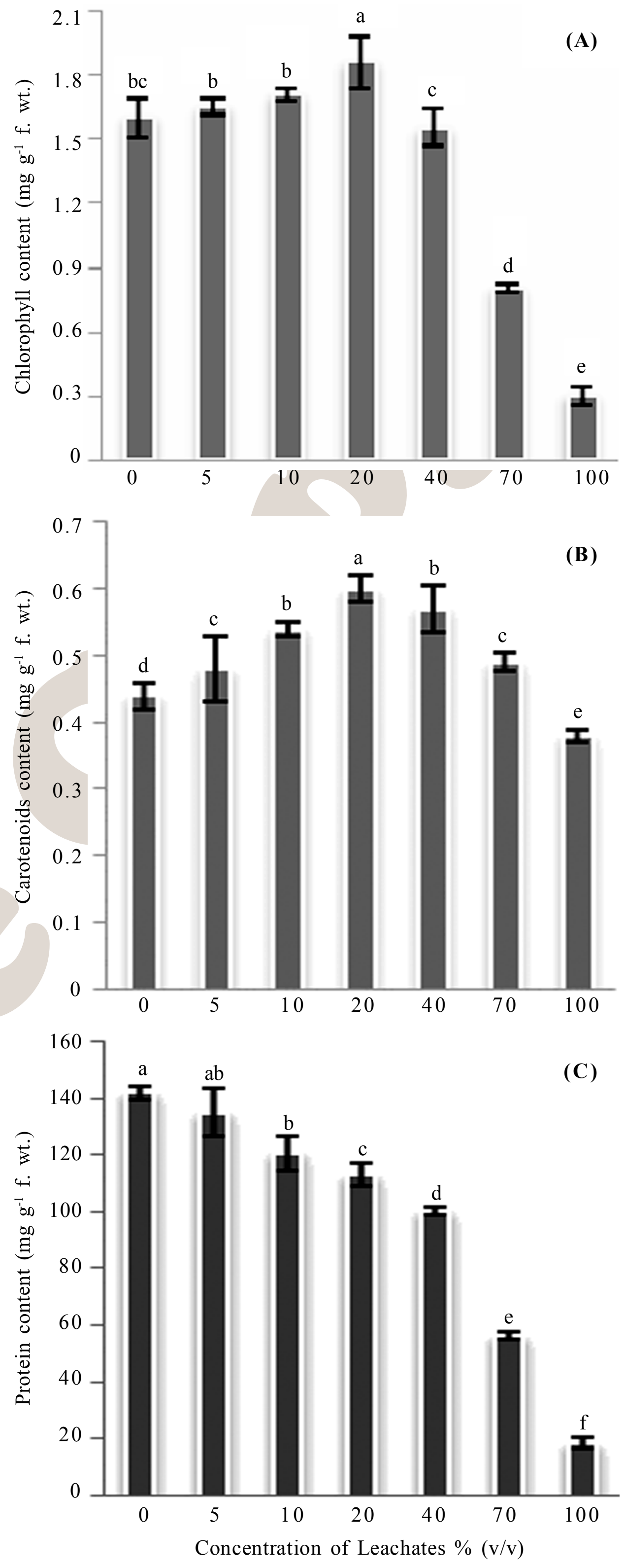

Fig. 1 : Effects of leachates on the (A) chlorophyll, (B) carotenoid and (C) protein content of *Pisum sativum.* Value are mean of three replicates ±S.E. shown by vertical bars.Values shown by letters are significantly different $p< 0.05$

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