Irrigational impact of Rubber Factory effluent on elemental bioaccumulation and metabolite concentration in component parts of *Pisum sativum* var. *auricle*

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The effluent discharged from Rubber Factory brought about deterioration in water quality. pH (4.2-9.4) showed fluctuation. Other parameters (mg/liter), viz., D.O. (1.7-5.6), B.O.D. (31.34-1291.67), C.O.D. (61.0-1610.50), Chlorides (295-6060), Free CO_2 (70-250), Oil and Grease (17.0-67.6) exceeded tolerance limits. Brownish-black colour, foul odour and poor transparency (2-3.5cm) indicated poor water quality. T.S.S., T.D.S. and heavy metals like Cr, Pb, Zn, Fe and minerals like Na, K, Ca, Mg, SO_4, PO_4 and Total Nitrogen concentration indicated organic and inorganic loading. Percentage of Ca, K, PO_4 and Total Nitrogen, Crude Protein and Ether Extract was significantly low in the seeds of effluent treated cultivar (*Auricle*) of *Pisum sativum*. On the contrary, concentration of Na, Fe, SO_4, Total Carbohydrates, Total Ash and Chloride were significantly higher.

Key -words – Rubber effluent, Pollution, Chemical composition, *Pisum sativum* var. *auricle*.

INTRODUCTION

THE indiscriminate disposal of wastes by large number of industrial units has led to rapid deterioration in the quality of aquatic environment at Bareilly. The effluent from Rubber Factory located at Fatehganj, Bareilly is disposed off through several drains which carry their pollution load into river Shankha that ultimately causes pollution in river "Ram Ganga" flowing at 8 km west from Bareilly. Cultivators of adjacent villages irrigate their crops with the polluted water of the factory. This has resulted in colossal damage to their crops. Several workers have studied the effect of industrial effluents on growth, yield and chemical composition of various crops (Tripathi, 1978; Bahadur & Sharma, 1990; Somashekar *et al.*, 1984; Somashekar *et al.*, 1992; Juwarkar *et al.*, 1993; Saini *et al.*, 1993; Sharma & Habib, 1994). No efforts seem to have been made to study the water pollution caused by Rubber Factory, Bareilly and its irrigation impact on mineral bioaccumulation and metabolite concentration in different parts of crop plants. An attempt has, therefore, been made to fulfil the above objective.

MATERIAL AND METHOD

The effluent disposed off from Rubber Factory was collected from its discharge points at weekly intervals and was analysed according to A.P.H.A. (1980). Quantitative estimation of heavy metals, viz., Cd, Cr, Cu, Pb, Ni, Fe, Zn, Co, Mn in the effluent was made by using Atomic Absorption Spectrophotometer (AAS 300 Parkin & Elmer). The data are presented in table-1.

Chemical analysis of root, stem, leaf and seeds was done according to Piper (1966). Seeds of *Pisum sativum* var. *auricle* were separately sown in unglazed earthen pots (22cm dia.) containing uniform density of garden loam soil mixed with farm-yard manure.

Thinning was done after one week of seedling emergence to permit one seedling to grow in each pot. The control and treatment sets were maintained with tap water and effluent, respectively.

Plants were harvested at the time of seed ripening after 135 days of sowing. Different parts, viz., root, stem, leaf and seeds were collected from the plants of control and treated sets and analysed for total ash, Na, K, PO_4^{-}P, Total N, Fe, SO_4, Cl, Crude protein, ether extract and total carbohydrates.Values are expressed in terms of dry weight percentage. Mean values ± S.E. were recorded for each parameter. Variance ratios, Critical differences and SEm ± were worked out according to Snedecor and Cochran (1967). The soil used in the control and treated sets was the same and was found to have pH (7.3), Nitrogen (1.42 kg ha^{-1}), Potassium (290 kg ha^{-1}), EC_e EC (0.8 mSc m^{-1}), Organic Carbon (1.65%), Organic matter (4.2%), WHC (42.13%), moisture (9.6%), sand (56%), silt (14%) and clay (30%).

DISCUSSION

The data (Table-1) indicated the brownish-black colour, foul odour and poor transparency (2-3.5 cm) of the effluent. pH (4.2 to 9.4) is not conducive for inhabitation and survival of aquatic life. The concentration of TDS, TSS, DO, BOD, Total Alkalinity, Total-N, Ca, Mg, Na, K, EC_e (m Scm^{-1}), Total Hardness, Dissolved Silica, Free CO_2, Cl, SO_4, PO_4, Oil and Grease, Cd, Cr, Cu, Pb, Ni, Fe, Zn, Co, Mn exceeded the permissible limits recommended by IS:2490.

Table 1. Physico-chemical parameters of effluent and Tap Water used in Seed Germination and Irrigational Treatments (Nov. 1990-April, 1991).
All unless specified are in mg/l

	Parameters	Effluent	Tap Water
1.	Colour	Brownish-black	Colourless
2.	Odour	Foul smelling	Odourless
3.	Temp. (°C)	18.5-30.00	20.2-32.6
4.	pH	4.2-9.4	7.0-7.3
5.	Transparency (cm)	2.0-3.5	100
6.	T.D.S.	323-1120	96-152
7.	T.S.S.	169-187	17-29
8.	DO	1.7-5.6	10.0-13.3
9.	B.O.D. 5 days 20°C	31.34-1291.67	2.5-3.0
10.	COD	61.0-1610.50	40.45
11.	Total Alkalinity (as $CaCO_3$)	48.3-330.10	13-62
12.	Total-N	8.8-63.0	-
13.	Ca	66.5-89.2	1.3-4.6
14.	Mg	41.3-57.9	9.6-14.2
15.	Na	15.9-21.5	2.0-2.4
16.	K	17.0-17.8	1.7-2.1
17.	ECe ($mScm^{-1}$)	3.9-4.8	1.3-1.7
18.	Total Hardness (as $CaCO_3$)	41.3-529.3	98-120
19.	Dissolved Silica	0.4-1.5	-
20.	Free CO_2	70-250	1.2-2.5
21.	C1	295-6060	19.4-23.2
22.	SO_4	110-144	1.6-4.8
23.	PO_4	1.2-4.7	0.2-0.4
24.	Oil & Grease	17.0-67.6	
25.	Heavy metals		
	Cd	0.02-0.3	-
	Cr	0.3-0.5	-
	Cu	0.5-0.8	-
	Pb	0.2-0.3	0.09
	Ni	0.3-0.5	-
	Fe	2.5-2.8	0.3
	Zn	3.0-5.5	1.2
	Co	0.3-0.5	-
	Mn	0.7-0.8	0.5

Calcium - There was overall decrease in the concentration of Calcium in all the components of *Pisum sativum* var. *auricle.* The percentage decrease in the root, leaf, seed and stem being 76.67%, 84.16%, 85.88% and 89.76%, respectively (Tables 2-5). Calcium in the form of Calcium Pectate is an important constituent of middle lamella. Its poor concentration in vegetative parts of the treated plants could be due to its poor intake as Ca^+ ions through plasma membrane. Precipitation of Calcium as Calcium Hydroxide and Calcium Carbonats in the soil seems to be important causative factor responsible for its restricted availability. Its deficiency resulted in thin week stems, poor development of leaves and poor Calcium content in seeds as reported by Clarkson and Hanson (1980).

Sodium - Sodium concentration increased significantly in all the four parts, maximum increase (592.30%) being in the case of root followed by seed (315.78%), stem (252.38%) and leaf (242.85%) (Tables 2-5). Higher Na content in the stem of treated crops may be attributed to rapid intake of Na^+ ions. Daubenmire (1970) reported that Na^+ ions in the soil solution inhibit the entry of K^+ ions. However, Sodium ions in association with Cl^- ions cause particles of plasma membrane to separate and enhance permeability (Bains & Fireman, 1968). Accumulation of Na^+ ions in association with weak and strong anions alter the pH of the soil solution and affect crop growth (Tripathi, 1978).

Potassium - Potassium content was poor in all the component parts. Decrease was maximum (95.68%) in stem of the treated crop followed by seed (78.87%), root (75.00%), and leaf (48.05%) (Tables 2-5). Potassium occurs in the plant cells only in the ionic form as micronutrient. Its poor availability was observed in the form of deficiency symptoms, viz., mottled chlorosis of leaves, necrotic areas of the tip and margins of leaves and shortening of internodes. As an activator of enzyme ALA dehydrase it has been reported to play an important role in the biosynthesis of chlorophyll (Tribe & Whittaker, 1972). Potassium has a marked effect on the weight of the seeds hence maximum reduction in the seeds may be attributed to its deficiency. Low pH has

Table 2. Chemical constituents of root in *P. sativum* var. *auricle* as effected by industrial effluent.

Parameters %	Control Mean± SE	Treatment Mean ± SE	% Decrease/ Increase	F	CD	SEm ±
Ca	0.06 ± 0.03	0.01 ± 0.008	- 83.33	59.33 ***	0.02	0.003
Na	0.01 ± 0.007	0.09 ± 0.05	+800.00	182.00 ***	0.002	0.0003
K	0.12 ± 0.07	0.03 ± 0.02	-75.00	125.00 ***	0.04	0.004
PO_4-P	0.09 ± 0.05	0.08 ± 0.04	-11.11	12.00 **	0.009	0.0014
Fe	0.002 ± 0.0001	0.02 ± 0.001	+900.00	246.50 ***	0.005	0.0008
SO_4	0.17 ± 0.98	0.22 ± 0.13	+29.41	11.50 **	0.06	0.009
Cl	0.32 ± 0.19	0.80 ± 0.46	+150.00	494.28 ***	0.10	0.01
Total-N	1.28 ± 0.74	1.36 ± 0.78	+6.25	13.71 **	0.07	0.011
Crude Protein	8.00 ± 4.62	8.50 ± 4.91	+6.25	13.71 **	0.44	0.067
Total Carbohydrates	82.46 ± 47.61	81.43 ± 47.02	-1.24	24.87 ***	0.68	0.103
Ether extract	0.09 ± 0.06	0.07 ± 0.04	-22.22	24.79 ***	0.015	0.0023
Total ash	9.36 ± 5.46	9.99 ± 5.77	+6.73	27.45 ***	0.334	0.051

** $P < 0.01$

*** $P < 0.001$

been reported to cause Potassium deficiency and adversely affects Nitrogen metabolism (Sinha & Mehta, 1992).

Phosphate - Decrease in phosphate content was in the order of seed> leaf> stem> root. Maximum decrease (42.33%) was in the seeds of treated crop. Soil pH has a direct bearing on intake of phosphates which become poorly available in pH range from 4 to 6.5 and 7.5 to 8.5. Higher intake of Sodium ions exerts toxic effect (Daubenmire, 1970). Nutrient imbalance is the main factor inhibiting growth (Brvok, 1983). Poor intake of Phosphate affected synthesis of protein, carbohydrates and fat (Tables 2-5).

Iron - Percentage increase in iron content in seeds, stem, leaf and root was 191.17%, 171.60%, 119.78% and 12.00%, respectively (Tables 2-5) Iron content in the effluent was also much higher than recommended tolerance limit. Due to low pH of the effluent for major span of crop growth iron became excessively soluble and beyond its judicious micronutrient limit it exerted toxic effect on ferredoxin which plays key role in nitrogen metabolism (Beevers, 1979). Inhibition in growth has also been found to be associated with suppression of the activity of saveral enzymes like peroxidase, catalase and of cytochrome. Bio-accumula-

Table 3. Chemical constituents of stem in *P. sativum* var. *auricle* as effected by industrial effluent.

Parameters %	Control Mean ± SE	Treatment Mean ± SE	% Decrease/ Increase	F	CD	SEm ±
Ca	0.21 ± 0.12	0.02 ± 0.01	-91.47	3286.68 ***	0.011	0.002
Na	0.02 ± 0.01	0.07 ± 0.04	+250.00	88.00 ***	0.002	0.0002
K	0.13 ± 0.08	0.06 ± 0.03	-53.84	107.78 ***	0.02	0.003
PO_4-P	0.13 ± 0.07	0.11 ± 0.06	-15.38	14.54 **	0.02	0.003
Fe	0.008 ± 0.005	0.02 ± 0.01	+150.00	399.74 ***	0.002	0.0003
SO_4	0.183 ± 0.11	0.32 ± 0.19	+ 74.86	29.63 ***	0.08	0.013
Cl	0.30 ± 0.17	0.62 ± 0.36	+106.66	232.85 ***	0.10	0.01
Total-N	1.51 ± 0.87	1.40 ± 0.81	- 7.28	7.89 **	0.13	0.02
Crude Protein	9.43 ± 5.45	8.75 ± 5.06	-7.21	7.89 **	0.81	0.122
Total Carbohydrates	82.70 ± 47.78	82.93 ± 47.88	+0.27	8.45 **	0.26	0.04
Ether extract	1.14 ± 0.66	1.03 ± 0.58	-9.64	12.66 **	0.10	0.015
Total ash	6.72 ± 3.88	7.29 ± 4.21	+8.48	123.38 ***	0.17	0.025

*** $P < 0.001$

** $P < 0.01$

Table 4. Chemical constituents of leaf in *P. sativum* var. *auricle* as affected by industrial effluent.

Parameters %	Control Mean ± SE	Treatment Mean ± SE	% Decrease/ Increase	F	CD	SEm ±
Ca	0.12 ± 0.07	0.01 ± 0.01	-91.66	41.19 ***	0.05	0.008
Na	0.02 ± 0.01	0.09 ± 0.05	+350.00	700.00 ***	0.02	0.001
K	0.15 ± 0.09	0.08 ± 0.04	-46.66	55.62 **	0.07	0.005
PO_4-P	0.17 ± 0.09	0.10 ± 0.06	-41.17	78.76 ***	0.107	0.016
Fe	0.009 ± 0.01	0.02 ± 0.01	+122.22	14.18 **	0.009	0.001
SO_4	0.18 ± 0.10	0.34 ± 0.20	+88.88	1130.47 ***	0.016	0.008
Cl	0.23 ± 0.14	0.53 ± 0.31	+ 130.43	61.50 ***	0.17	0.018
Total-N	1.82 ± 1.05	1.48 ± 0.85	- 18.68	216.75 ***	0.076	0.011
Crude Protein	11.37 ± 26.57	9.25 ± 5.30	-18.64	216.75 ***	0.48	0.07
Total Carbohydrates	79.31 ± 45.85	80.75 ± 46.68	+1.81	31.08 ***	0.698	0.106
Ether extract	1.20 ± 0.69	1.11 ± 0.64	-7.50	10.56 **	0.091	0.010
Total ash	8.01 ± 4.62	8.79 ± 5.08	+9.73	25.06 ***	0.51	0.08

** $P < 0.01$
*** $P < 0.001$

tion of Fe in plants at low pH is amply documented (Bose & Singh, 1991).

Sulphates - Concentration of sulphates has been found to be significantly higher in all the parts of the treated crop,maximum being in the seeds (95.43%) followed by leaf (93.33%), stem (75.41%), root (17.65%). Sulphate content was also higher in the effluent as compared to tap water. Sulphates are mostly soluble in water and impart hardness. Sulphates along with other inorganic solutes are absorbed through plasma membrane and become important constituent of proteins and vitamins like biotin,thiamine and coenzyme A. In association with cations like Ca^{++} and Mg^{++} they bring about lowering of pH in soil solution.

Under reducing anaerobic conditions they are reduced to sulphides and may also lower down the pH and exert adverse effect on plant metabolism (Steiver, 1967).

Chloride - Chloride percentage increased significantly in the all parts of the treated plants. Percentage was maximum in seeds while minimum (124.12%) values are obtained in the case of leaf. Chloride content was higher in treated crops (Tables 2-5). Alongwith

Table 5. Chemical constituents of seed in *P. sativum* var. *auricle* as affected by industrial effluent.

Parameters %	Control Mean ± SE	Treatment Mean ± SE	% Decrease/	F	CD	SEm ±
Ca	0.08 ± 0.05	0.01 ± 0.007	- 87.50	380.64 ***	0.012	0.002
Na	0.01 ± 0.06	0.07 ± 0.04	+600.00	40.09 ***	0.02	0.002
K	0.95 ± 0.54	0.10 ± 0.06	-89.47	27035.00 ***	0.15	0.01
PO_4-P	0.30 ± 0.17	0.17 ± 0.09	-43.33	19.14 **	0.096	0.014
Fe	0.003 ± 0.002	0.02 ± 0.009	+566.66	83.15 ***	0.005	0.0008
SO_4	0.21 ± 0.13	0.42 ± 0.25	+104.76	225.54 ***	0.009	0.007
Cl	0.13 ± 0.08	0.89 ± 0.51	+584.61	282.00 ***	0.21	0.02
Total-N	3.58 ± 2.07	2.31 ± 1.33	- 35.47	531.73 ***	0.182	0.027
Crude Protein	22.37 ± 12.90	14.43 ± 8.34	-35.49	531.71 ***	1.13	0.17
Total Carbohydrates	73.29 ± 42.32	81.09 ± 46.79	+10.64	495.66 ***	1.15	0.175
Ether extract	1.73 ± 0.99	1.24 ± 0.72	-28.32	360.15 ***	0.085	0.013
Total ash	2.60 ± 1.50	3.23 ± 1.86	+24.23	595.35 ***	0.085	0.013

** $P < 0.01$
*** $P < 0.001$

essential anions like SO_4^-, NO_3^-, Chloride ions were also taken up through plasma membrane and got accumulated in cytoplasm. Under judicious limits chlorides are required in catalytic amounts to carry on various enzymatic reactions in the cells. However, when all the negative charges on the particles of protoplasm are neutralised and substituted by negative Cl^- units the permeability of plasma membranes is at the maximum. Chloride ions are among strong anions that increase toxicity both in water and the cell sap (Kudesia, 1980).

Total Nitrogen - Total Nitrogen exhibited significant decrease in treated plants over the respective control. The decrease was in the following sequence- 35.47%, 18.68%, 7.28%, 6.24% in seeds, leaf, stem, and root, respectively. Though the concentration of nitrates and nitrites was higher in effluent the reduction in the total nitrogen in the seeds suggests impairement of nitrification caused by inactivation of microbes at low pH (6.5-4) of the effluent (Tables 2-5). Effect of nitrogen starvation was manifested as yellowing of leaves brought about by reduction in Chlorophyll biosynthesis and depressive effect on nitrogenous bases like,purines and pyrimidines as observed by Sinha *et al.* (1988).

Total Carbohydrates - Data revealed overall increase in the concentration of total carbohydrates in the root, stem, leaf, and the seeds of the cultivars studied. Maximum increase (10.64%) was obtained in the case of seed followed by leaf (1.81%), root (1.24%) and stem (0.27%). Greater concentration of carbohydrates was found to be associated with decrease in fat content. Regeneration of organic matter in the form of carbohydrates takes place in a cyclic process in which CO_2, H_2O and O_2 which are liberated by the decomposing organic matter become readily available for their synthesis as opined by Caputto *et al.* (1967).

Crude Protein - Crude protein contents exhibited overall decrease in seed, leaf, stem and root, being 35.47%, 18.68%, 7.28% and 6.25% respectively. Protein content has been found to be positively correlated to total-N. Inorganic nitrogen is taken up as NO_3^- ions is converted into NH_2 groups before being elaborated into amino acids (Boulter, 1970). Synthesis of proteins as also of fats is intimately linked with carbohydrate metabolism. Protein breakdown into amino acids is also adversely affected due to effluent toxicity (Beevers, 1979). Hence poor availability of nitrogen may be a causative factor for reduction in crude protein content in different parts. Decrease in protein content has been found to be associated with increase in total carbohydrates which is nutritionally unsound since with decline in protein the subtle balance of amino acids is disturbed (Tables 2-5).

Ether Extract - Ether extract showed overall decrease in all the component parts of the treated crop (Tables 2-5). Maximum decrease was observed in the case of seeds followed by root, stem and leaf over their respective controls. This is attributed to decline in carbohydrate reserves leading to break down of fats that are first hydrolysed in the presence of lipases to yield fatty acids and glycerol. Suppression of fat metabolism is accounted by inhibitory action of heavy metals,sodium and chlorides on fatty acid synthesizing enzymes (Webb, 1966).

Total Ash - Percentage of ash content was higher in the seeds of treated cultivar as compared to control (Tables 2-5). Ash content of the cultivar is the direct manifastation of bioaccumulation of serveral minerals utilized for synthesis of proteins, carbohydrates, lipids. Some become important constituents of protoplasm and cell wall while others enter as components of large number of enzymes and, also that of chlorophyll. Excessive solubility of Zn, Mn, Pb, Cr, Fe, and Cu at low pH (4 to 6.5) is the chief factor creating toxicity as opined by Singh and Mukhiya (1980). Intake of toxic metallic ions results in their bioaccumulation in plant tissues. Metabolic attributes such as lipids, crude proteins and total carbohydrates being dependent on the efficiency of enzymes and intake of minerals from soil thus exhibited variation in their concentration. Soil pH plays a decisive role by governing their intake. It is, therefore, desirable to ameliorate the pH of effluent without much alteration in EC levels and treatment designs be modified according to the nature of prevailing pollution.

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