

### 3. MATERIAL AND METHODS

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The soil incubation and pot culture experiments were conducted at the Department of Chemistry, N.R.E.C. Post-Graduate College Khurja (Bulandshahr), U.P. under the investigation entitled “Remediation of heavy metal contaminated soils using different amendment and different *species of Brassica*”. The incubation experiment aimed to study the changes occurring in DTPA and diacid extractable fraction of heavy metals (Zn, Cu and Ni) in soil at 6 and 12 month intervals following the incorporation of soil. The influence of amendments, namely FYM (Farm yard manure), lime ( $\text{CaCO}_3$ ), Single super phosphate (SSP) and combination FYM+ $\text{CaCO}_3$ , on soil moisture regimes in the incubation study. The pot culture experiments involved growing of different plant species viz. *B. juncea*, *B. campestris*, *B. carinata*, *B. napus*, *B. nigra* in heavy metals and sewage effluents treated soil with the addition of amendments (FYM,  $\text{CaCO}_3$ , SSP and  $\text{CaCO}_3$ +FYM), which were conducted with the aim to compare the five crops in relation to the accumulation of Zn, Cu and Ni in influence of amendments on the uptake of heavy metals by these crops.

#### 3.1 Location, climate and weather conditions:

Khurja town is located at  $28^\circ 14' \text{ N}$  latitude and  $77^\circ 50' \text{ E}$  longitude at an altitude of about 275 metres above the mean sea level. The town falls under sub-tropical zone characterized by hot dry summers and cold winters. The mean summer and winter temperature are  $30^\circ \text{ C}$  and  $13^\circ \text{ C}$ , respectively with wide diurnal variations. The mean annual precipitation is from 80 to 90 cm. most of which is normally received through south-west monsoon during July to September.

#### Soil sampling:

Bulk surface sample (0-15 cm) of soil (Typic Haplustep) was collected from the agricultural land around Khurja town (Bulandshahr) U.P. The processed soil sample was used for laboratory and pot culture studies. Some important physico-chemical characteristics of the experimental soil have been shown in table 2a.

### **Incubation Experiment:**

The soil incubation experiment was conducted in plastic pot of 4 kg capacity. The experiment had five treatments, comprising of control, FYM, SSP, CaCO<sub>3</sub> and FYM+ CaCO<sub>3</sub>. The soil experiment was conducted in pots to study the application of Zn, Cu and Ni as well as different amendments in metal contaminated soil. A basal dose of 45 N and 25 K<sub>2</sub>O (mg kg<sup>-1</sup> Soil) was added in the form of urea and muriate of potash. After incorporation of basal nutrients in solution form, metal was applied at the rate of 0 and 20 Zn +10 Cu +2.5 Ni (mg kg<sup>-1</sup> soil) in the form of hydrated salts of concerned metals viz. ZnSO<sub>4</sub>.7H<sub>2</sub>O, CuSO<sub>4</sub>.5H<sub>2</sub>O and NiSO<sub>4</sub>.6H<sub>2</sub>O. Then the soil was thoroughly mixed with different amendments viz. control, FYM (1%), SSP (332 mg kg<sup>-1</sup> soil), CaCO<sub>3</sub> (5%) 50g kg<sup>-1</sup> soil and FYM (1%) +CaCO<sub>3</sub> (5%). Deionized water was added to bring the soil to field capacity and the soil was incubated for one week. Each treatment was replicated thrice.

**Soil sampling:** Soil samples from the incubation experiment from submerged soil conditions were drawn at 6 and 12 months after treatment application. The soil samples were first dried in air in oven at 70°C till constant weight, DTPA and diacid extractable heavy metal elements in soils was estimated.

**DTPA extractable heavy metals:** Zn, Cu and Ni in soil were determined by DTPA. Soil was extracted with DTPA solution for available Zn, Cu and Ni as outlined by Lindsay and Norvell (1978). Extracting solution consists of 0.005 M DTPA, 0.01 M CaCl<sub>2</sub>.H<sub>2</sub>O and 0.1 M triethanolamine (TEA) and the pH was adjusted to 7.2 ± 0.05.

To extraction, to 10 g air dried soil in polythene bottle, 20 ml of extractant was added and the contents were shaken for 1-2 hours. After filtration the extracts were analyzed for Zn, Cu and Ni with flame Atomic Absorption Spectrophotometer (AAS) at the department of soils, Punjab Agricultural University, Ludhiana, (Punjab).

**Diacid extractable heavy metals:** Soil sample were digested with diacid mixture (hydrofluoric and perchloric acids) in a platinum crucible and subsequently the contents were dissolved in 6 N HCl as per the procedure of Jackson (1967). Zn, Cu and Ni contents in the digests were determined with flame Atomic Absorption Spectrophotometer (AAS) at the department of soils, Punjab Agricultural University, Ludhiana (Punjab).

## **Treatment details:**

### **1. Metal levels: Two**

- a) Nil
- b) 20mg Zn + 10 mg Cu +2.5 mg Ni per kg of soil.

### **2. Amendments : Five**

- a) Control
- b) Farm yard manure (FYM):- Well rotten and dried farmyard manure was added at the rate of 10 g per kg of soil (1%)
- c) Single super phosphate (SSP):- Single Super Phosphate was added at the rate of 120kg P<sub>2</sub>O<sub>5</sub> per hectare. For this, 332 mg of SSP was added per kg of soil.
- d) Calcium Carbonate :- CaCO<sub>3</sub> was added at the rate of 50 g per kg of soil (5%)
- e) FYM and CaCO<sub>3</sub> :- FYM and CaCO<sub>3</sub> were added together at the rate of 10 g and 50 g per kg of soil respectively.

### **3. Soil sampling stages : Two**

- a) 6 Months after treatment application.
- b) 12 Months after treatment application.

### **4. Replications : Three**

### **5. Experimental design :- Completely Randomized Design (Factorial)**

### **6. Treatment Combinations :**

- a) Control.
- b) 20 mg Zn+10 mg Cu + 2.5 mg Ni per kg of soil.
- c) 20 mg Zn+10 mg Cu + 2.5 mg Ni per kg of soil.+ Farm yard manure (FYM)
- d) 20 mg Zn+10 mg Cu + 2.5 mg Ni per kg of soil. + Single Super Phosphate (SSP)
- e) 20 mg Zn+10 mg Cu + 2.5 mg Ni per kg of soil. + Calcium Carbonate (CaCO<sub>3</sub>).
- f) 20 mg Zn+10 mg Cu + 2.5 mg Ni per kg of soil. + FYM + CaCO<sub>3</sub>.

**Pot culture experiment:**

Pot culture experiment with *Brassica species* was conducted at the department of chemistry, N.R.E.C. College, Khurja (Bulandshahr), U.P. during the season of 2009-10.

Four kg of processed soil was filled in each of the 90 plastic pots (Capacity, 5 kg) and required amount of amendments and added metals, according amount to the treatments, was added and mixed in pots the last week of August, 2008. Water was added to each pot and kept standing 1 cm. above the soil surface to have the submerged soil moisture condition up to 12 months period and pots soil contents were mixed thoroughly with wooden stick.

**Pot culture experiments with *Brassica species* (rabi, 2009-10):**

Pot culture experiments with *Brassica speices* were conducted in five sets of 5 kg capacity (Per sets in 18 pots), pots in the net house at the department of chemistry, N.R.E.C. College, Khurja (Bulandshar), U.P.

**Crop:** Five different *species of Brassica*

1. *Brassica juneca* (Indian mustard)
2. *Brassica campestris* (Yellow sarson)
3. *Brassica carinata* (Ethiopian mustard)
4. *Brassica napus* (Gobi sarson)
5. *Brassica nigra* (Banarasi rai)

**Replication:** Three

**Experimental Design:** Completely Randomized Design (Factorial).

**Growing the crop :** After thorough mixing of the soil with amendments and heavy metals. Pot filling was done and the seeds of *Brassica* were sown. A handful of soil was removed from each pot, about eight to ten seeds of the respective species were spread uniformly in each pot and subsequently were covered with soil. Ten to fifteen days after germination seedling were thinned out to maintain four plants per pot.

**Harvesting of *Brassica species* :** The *Brassica species* in all sets of pots were allowed to grow up to full flowering stage. Irrigation was done as and when required to maintain the moisture at field capacity. All the crops (*Brassica species*) were harvesting at full blooming stage, first dried in air and then in hot air up to at 65°C till the constant weight. Drymatter yield of each species per pot was recorded.

**Plant Analysis:** The oven-dried plant sample was 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  $\text{HNO}_3$  and later digested with diacid mixture of  $\text{HNO}_3$ :  $\text{HClO}_4$  (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 Zn, Cu and Ni using flame Atomic Absorption Spectrophotometer (AAS) at the Department of soils, Punjab Agricultural University, Ludhiana, (Punjab).

### **Statistical Analysis**

Analysis of data generated by incubation experiment as well as pot culture experiment was followed to elucidate the effect of various amendments on availability of metals in soils. Factorial Completely Randomized Design (CRD) was used for evaluating the main effect as well as interaction effects of the three factors, species, type of soil and physiological stages of growth.