Materials

and

Methods
3.1 Soil sampling

Soil is inherently a heterogeneous material and its chemical and physical nature varies from place to place, both laterally and vertically. Variations in topography, in farming operations, in soil type, in drainage, and in geochemical parent material or history can occur within the field under consideration. The first task is therefore to obtain a soil sample that is representative of the field or other area under consideration.

Since the purpose of any soil survey is to characterize the area as fairly as possible within the limitation of the number of samples that it is possible to take and analyze, the efficiency of the sampling plan is of major concern. The soil of no region, however small, is uniform and observations must be replicated if the average is to be estimated at all precisely. Replication is expensive, and so for many years investigators have wanted to know how much sampling they should do to achieve sufficiently precise estimates.

Systematic regular sampling and random samplings are the two principal sampling patterns recommended for soil sampling. Systematic sampling i.e. at regular intervals along a transect or on a grid give the most precise estimates of a given parameter. Webster (1977) has also demonstrated the advantages of sampling on grids rather than simply at random. Most of the soil sampling plans rely on composite samples on the assumption that the results from the composite samples are representative of the sampling units contributing. Compositing is valid only if:

- The sampling volume represents a homogeneous population
- Equal amounts of each sampling unit contribute to the subsample analyzed
- No interaction that would affect the results materially occur
- An unbiased estimate of the mean is the only objective.

Soil sample is the least well defined of all the stages of soil analysis, and its importance often ignored, is fundamental, since the variation in soil properties can be very large over quite small distances on the ground. Sampling error is much greater than analytical errors for soils. In view of the variability of soils, it seems impossible to devise an entirely satisfactory method of sampling. It is obvious that
details of the procedure should be determined by the purpose for which samples are taken.

Characteristics of the soil profile depend not only on the parent rock (or transported till) material but also on climate, topography and biological activity. In semiarid warm climates less extensive downward leaching occurs, often producing calcareous soils with the formation of a caliche layer (CaCO₃ precipitation) (Hall, 1998). The generalized soil profile of arid (desert) environments usually has only C and D horizons. D horizon consists of only bedrock and C horizon is the zone of weathering bedrock, loose and partly decayed.

The soil sampling and processing includes collecting a representative soil sample and further pretreatment (air-, freeze-, oven-drying) storage and processing the sample for final analysis. Chemical properties of soils vary not only vertically, horizontally, and with pretreatment but also with time. When soil is dried, vital information on speciation may be lost (Ure et al., 1996). However, the use of field moist samples presents some practical difficulties. Moist soil is more difficult to homogenize (and hence, to sample representatively) and, where immediate extraction is not possible, microbial and chemical reactions may lead to interspecies transformations. Redistribution of element species on drying (air-, oven-, freeze-) well aerated samples is of less concern than for anoxic samples, but never the less the storage of samples between collection and analysis remains an area of further investigation (Kersten and Forstner, 1989).

3.1.1 Sampling strategy

As the objective of the sampling was to study soil-trace element interaction, and not to derive a concentration map, the sampling strategy followed was judgmental, thus samples were chosen to be representative of entire range of solid matrices found in the affected area. In this area, there is a high degree of heterogeneity because of the presence of different types of solid matrices. Where possible, non-contaminated as well as highly contaminated matrices were taken from “hot spots”. For the purpose of this study the seven important locations based on different anthropogenic activities were selected for sampling (Fig.3.1). The sampling locations were:

1. Old tailing dam: Area where the tailings prior to 1980 were dumped and now has been stabilized by plantation (n = 4, 0-40cm).
2. New tailing dam: Area used presently for tailing impounding (n = 5, 0-50 cm)
3. Old Zawar village: Ancient smelting village which has been abandoned now and is an heritage site (n = 4)
4. Balaria mine area: Functional mining (n = 22)
5. Mochia mine area: Functional mining (n = 21)
6. Zawarmala mine area: Functional mining (n = 18)
7. Baroi Mine area: Closed mine (n = 26)

All the seven locations were further divided into grids of size 250X250 m. From location no. 3 to 7 most of the samples were collected up to a depth of 10 cm, except a few where the sampling depth was up to 20 cm. In addition to these samples, mining wastes (n = 3), old smelting slags (n = 4) and soils near old workings/derelict surface mines (n = 4) were also collected to have a detailed picture of the area, in terms of metal levels and their relative mobilities.

3.1.2 Sample preparation

In the present study, soil samples were collected as undisturbed core, by using a specially designed cylindrical stainless steel corer. It was tried to sample the soil core up to the maximum possible depth. But, as the soil cover in most of the grids was shallow, the soil samples were collected up to a depth of 10 cm. The tailings were sampled up to a depth of 50 cm in the new tailing dam and till 40 cm from the old tailing dam. As no visible distinction between different layers was observed, the soil cores were divided into layers of 10 cm each. Soon after collection, the soil samples were carefully transferred to clean and dry self-sealing polyethylene bags for transport to laboratory. The whole soil was air dried for three weeks in paper lined propylene trays at room temperature. The dried material was then gently rolled with a wooden roller to break up large aggregates and sieved through 2 mm sieve. The sample was thoroughly mixed and homogenized by coning and quartering and stored in tightly sealed polyethylene bags until further analysis. Grinding was avoided, as it might effect the original nature and surface area of the sample and hence the measured metal levels in total and different fractions. It was considered preferable to analyze a larger sample mass instead of grinding and homogenizing, to decrease the effect of heterogeneity of the sample. All further analysis was carried out on sample <2 mm.
Fig. 3.1 Map of Zawar area showing study locations

LOCATION OF MINES
LONGITUDE: 73°40'-22" TO 73°45'-08"
LATITUDE: 24°18'-50" TO 24°22'-47"

INDEX
1. MINING LEASE BOUNDARY
2. FENCING
3. ROAD AND BUILDINGS
4. EFFLUENT COLLECTION CENTRE
5. RAILWAY TRACK
6. AIR MONITORING STATION
7. GRUTT-PLANTATION
8. MONITORING WELLS
9. RIVER
10. WASTE DUMPING AREA

HINDUSTAN ZINC LIMITED
(A GOVERNMENT OF INDIA ENTERPRISE)
ZAWAR MINES
ENVIRONMENT PLAN AND LAND USE OF ZAWAR MINES
SCALE 1:10000
All chemicals used in this study were of analytical grade, and double distilled or M.Q water was used during the course of experimentation. All extractions were conducted in triplicate in acid washed labware. Multi level standards were prepared for each extraction step in the same matrix as the extracting reagents to minimize matrix effects. Blanks were run simultaneously for background correction and other sources of error.

3.2 Physicochemical parameters

3.2.1 pH and Electrical conductivity (EC)

The pH and EC of the different solid matrices were determined in an aqueous suspension in the ratio (w:v), sample (1) : distilled water (2.5) (Okalebo et al., 1993). The pH meter was calibrated with the buffer solutions of pH 4.0, 7.0 and 9.2. 0.1 M KCl was used for calibration during conductivity measurement.

3.2.2 Total organic matter (TOM)

The organic matter of selective samples was determined by Walkley Black method. This method involves the oxidation of organic matter by oxidising agent added to the sample in excess, and the subsequent titration of the excess oxidising agent. The method is based on chromic acid oxidation.

3.2.3 Particle size analysis (PSA)

The soil texture is one of many soil properties used in soil survey to differentiate one soil from the other. It is a property produced by soil-forming processes and has great edaphological importance. The soil texture is defined with reference to the size distribution of particles that make up a soil.

Particle-size analysis (PSA) is a measurement of the size distribution of individual particles in a soil sample. The major features of PSA are the destruction or dispersion of soil aggregates into discrete units by chemical, mechanical, or ultrasonic means and the separation of particles according to size limits by sieving and sedimentation.

For the present study the USDA classification (i.e., sands (<2000-50 μm), silts (<50-2 μm), and clays (<2 μm) was adopted. PSA was determined by following the pipet method as described by Gee and Bauder (1986).
3.2.3 Carbonate content
Carbonates may be found in all the particle-size classes of soil, being either inherited minerals in the stone, sand—and silt-sized fractions, or a mixture of inherited and pedogenic minerals in the clay-sized fraction. They may also occur in arid regions cementing large volumes of soil into hardened material termed calcrite. Carbonates maintain alkaline conditions in soils and influence the growth of plants through the direct effect of dissolved bicarbonates and the indirect effect of high pH on the solubility and availability of nutrients, particularly phosphorus, copper, zinc, iron and manganese.

The carbonate content of soils was determined by following the method of Rowell (1994). This analysis involves the dissolution of carbonates in an excess of standard acid. It determines calcium and magnesium carbonates together, but is often expressed as an equivalent amount of calcium carbonate (CaCO₃), i.e. the amount of pure CaCO₃ that would have reacted with the acid used in analysis. The soil carbonates are reacted with standard hydrochloric acid (HCl) and the excess acid titrated with standard sodium hydroxide (NaOH).

3.3 Total metal analysis
The analysis of heavy metals in solid samples is one of the most dynamically developing scientific areas. The decomposition of samples with siliceous materials, such as soil and sediment is difficult to accomplish. Most of the methods for determination of major, minor, trace and ultra-trace elements in solid samples require a previous digestion of samples, a time consuming and a highly risky step, which can cause some volatilization loss of the elements to be determined or can lead to contaminations. Unfortunately, a generalized method for the pretreatment of solid samples can not be prescribed due to the diversity of materials to be digested, the nature of the elements to be determined and the analytical technique to be used for measurement. The five primary considerations in selection of a judicious sample decomposition procedure are: data quality, objectives, matrix, analyte and reaction properties.

Hot plate, pressure digestion (pressure bomb) and microwave assisted heating technique are widely used methods for solid matrix digestion prior to instrumental detection. Mineral acids such as HCl, HNO₃, HClO₄, H₂SO₄ and their mixtures have been used for the dissolution and extraction of metals from soils for
"Pseudo total" analysis. In most cases, complete digestion of the sample is required to achieve reproducible and accurate results. For "total elemental" analysis, the use of hydrofluoric acid in conjunction with other acid mixtures for the digestion of solid samples containing silicon is a well established conventional approach. Hydrofluoric acid is a non oxidizing acid whose reactivity is based on its strong complexing nature. It is most commonly used in inorganic analysis because it is one of the few acids that can dissolve silicates:

\[ \text{SiO}_2 + 6\text{HF} \rightarrow \text{H}_2\text{SiF}_6 + 2\text{H}_2\text{O} \]

If the silicon content in the sample is known, the amount of HF can be scaled accordingly. Following dissolution, many analyses require removal of all hydrofluoric acid to reduce the damage or to resolubilise insoluble fluorides. Either the excess of HF is fumed out or the fluoride ion is complexed with boric acid.

\[ \text{H}_2\text{SiF}_6 \rightarrow \text{SiF}_6(\text{g}) + 2\text{HF}(\text{g}) \]

The function of boric acid in the second stage of digestion is not only to mask the free fluoride ions in the solution, but also to facilitate the dissolution of free fluorides (Wu et al., 1996).

\[ \text{H}_3\text{BO}_3 + 3\text{HF} \rightarrow \text{HBF}_3(\text{OH}) + 2\text{H}_2\text{O} \]
\[ \text{HBF}_3(\text{OH}) + \text{HF} \rightarrow \text{HBF}_4 + \text{H}_2\text{O} \]

From the viewpoint of total decomposition, a tri-acid digestion method, using a combination of HClO₄, HNO₃ and HF described by Agemian and Chau (1975) was followed for the present work.

3.4 Chemical speciation and mobility assessment for cadmium, lead and zinc

Bioavailability coupled with affinity can be applied back to potential toxicity of the element and an assessment of potential hazard to the environment made, although according to Alloway and Ayers (1997) "it is difficult to generalize about toxicity". Methodological difficulties are also encountered in terms of
measuring of bioavailability since it is usually operationally defined. Apte and Gardner (1991) state that "complexation decreases toxicity". Use of total concentration as a criterion to assess the potential effects of metal contamination in the soil environment is not satisfactory. Elements are present in soil in various forms and these can strongly affect the behavior of the element within the soil in terms of its biological availability, potential toxicity, chemical interactions and mobility within the profile. Therefore, to gain more precise understanding of the potential and actual impact of elevated levels of metals in soil, it is necessary to identify and quantify the forms in which a metal is present in the soil.

The manner in which metals are bound to soils and sediments can exert a major control on their bioavailability, toxicity and biogeochemical cycling. Numerous selective extraction schemes have been used for discriminating individual geochemical phases, ranging from simplistic methods for differentiating "labile" and "residual" fractions (single extractions), to more complex approaches facilitating the sequential removal of adsorbed, carbonate, phosphate, Fe/Mn oxide, sulphide, organic and silicate fractions (sequential extraction procedures). In a sequential extraction selective reagents are used consecutively, each being more drastic in action or of a different nature than the previous one, to extract "operationally defined phases" from solid matrix. Comparing the results of two or more schemes on a batch of samples can generate additional information on the strengths and limitations of the methods, and if complementary leaching sequences are combined, then more valuable speciation data can be derived than could be achieved by use of single systems.

3.4.1 Single batch extraction

To assess the potential mobility of Cd, Pb and Zn in different kind of matrices, a single extraction scheme using EDTA as described by Quevaувiller et al. (1998) was adopted.

**EDTA extraction protocol**

0.05 mol/l EDTA was prepared as an ammonium salt solution by adding in a fume cupboard (146 ± 0.05)g of EDTA free acid to (800±20) ml distilled water and by partially dissolving by stirring in (130 ± 5) ml of saturated ammonia solution. The addition of ammonia was continued until all the EDTA has dissolved. The obtained solution was filtered if necessary through a filter paper of
porosity 1.4-2.0 μm into a 10 L polyethylene container and diluted with water to (9.0 ± 0.5) L. The pH was adjusted to (7.00 ± 0.05) by addition of few drops of either ammonia or hydrochloric acid as appropriate. The solution was thereafter diluted with distilled water to (10 ± 0.1) L, well mixed and stored in a stoppered container.

A 5g soil sample was transferred to an extraction bottle in which 50 ml of 0.05 mol/l EDTA was added. The obtained mixture was shaken on an end-over-end shaker operating at 30 rpm for 1 h at room temperature (20 ± 2)°C.

The extractant was separated immediately. A portion of the extract was decanted in a centrifuge tube and centrifuged for 10 min. at 3000Xg. The supernatant liquid was stored in a polyethylene container at 4° C.

3.4.2 Sequential extraction procedure

For this study, we selected the sequential extraction procedure proposed by the European Union’s Standards, Measurements and Testing Program (SM&T) (Sahuquillo et al., 1999) and further described by Rauret et al. (1999). All the vessels in contact with samples or reagents were cleaned by soaking in HNO₃ 4 mol/l (overnight) and rinsed repeatedly with distilled water. All the extractions were performed by using a mechanical end-over-end shaker at a speed of 30±10 rpm, 100 ml centrifugation tubes were used and centrifugation was done 3000g for 20 minutes to separate the phases.

Reagents

- **Water:** Glass double distilled water was used, simple de-ionised water may contain organically complexed metals and was not used.

- **Solution A (acetic acid 0.11 mol/l):** In a fume cupboard, (25 ± 0.2) ml of redistilled glacial acetic acid is added to about 0.5 l of distilled water in a 1 l polyethylene bottle and made upto 1 l with distilled water. 250 ml of this solution (acetic acid 0.43 mol/l) is made up with distilled water to 1l to obtain an acetic acid solution of 0.11 mol/l. A sample of each batch of solution A is analysed.

- **Solution B (hydroxylamine hydrochloride or hydroxylammoniumchloride 0.5 mol/l):** 34.75g of hydroxylamine hydrochloride is dissolved in 400 ml of distilled water and by means of a calibrated pipette 25 ml of 2 mol l⁻¹ HNO₃ is added. Final volume was made upto
1 l with distilled water. This solution is always prepared on the same
day as extraction is carried out.

- **Solution C (hydrogen peroxide solution 300mg/g, i.e. 8.8mol/l):** H₂O₂
  supplied by the manufacturer is used, i.e. acid-stabilized to pH 2-3.
- **Solution D (ammonium acetate 1 mol/l):** 77.08g ammonium acetate is
dissolved in 900 ml of distilled water, adjusted to pH 2 with HNO₃ and
made up to 1 l with distilled water.

**Sequential extraction procedure (modified BCR procedure)**

**Step 1 (B1 fraction):** 40 ml of solution A is added to 1 g of sediment in a 100 ml
centrifuge tube and extracted by shaking for 16 h at 20 ± 2 °C. There should be no
delay between the addition of extractant solution and the beginning of the shaking.
The residue is separated by by centrifugation and decantation of the supernatant
liquid. The extract is stored at 4 °C prior to analysis. The residue is washed by
adding 20 ml distilled water, shaking for 15 minutes and centrifuging. The
supernatant is decanted and discarded.

**Step 2 (B2 fraction):** 40 ml of solution B is added to the residue from step 1 and
extracted by shaking for 16 h at 20 ± 2 °C. The residue is separated by
centrifugation and decantation of the supernatant liquid. The extract is stored at
4 °C prior to analysis. The residue is washed by adding 20 ml distilled water,
shaking for 15 minutes and centrifuging. The supernatant is decanted and
discarded.

**Step 3 (B3 fraction):** 10 ml of solution C is added carefully, in small aliquots to
avoid losses due to possible violent reaction to the residue from step 2. The vessel
is covered with watch glass and digested at room temperature for 1h with
occasional manual shaking. The digestion is continued for 1 h at 85 °C and the
volume is reduced by further heating of the uncovered vessel in a steam bath. A
further aliquot of 10 ml of solution C is added. The covered vessel is heated again
to 85 °C and digested for 1h. The cover is removed and the volume of the liquid is
reduced to a few ml. 50 ml of extracting solution D is added to the cool moist
residue and shaken for 16 h at ambient temperature. The residue is separated by
centrifugation and decantation of the supernatant liquid. The extract is stored at
4 °C prior to analysis. The residue is washed by adding 20 ml distilled water,
shaking for 15 minutes and centrifuging. The supernatant is decanted and discarded.

**Step 4 (B4 fraction):** As an internal check on the procedure, the residue from step3 is digested using the HF - HNO₃ - HClO₄ dissolution procedure as used for the total metal extraction and the total amount of the metal extracted (i.e. sum of step 1 + step 2 + step 3 + residual) compared with that obtained by tri-acid digestion of a separate 1 g sample of the solid.

In addition, for the purpose of figuring out the applicability of different SEP's, two SEP's BCR and Tessier's were used to compare the metal fractionation data for mine tailings (New and Old tailings).

**Sequential extraction procedure (Tessier et al., 1979)**

For the present study the sequential extraction scheme designed by Tessier et al., (1979) was used. The extractant used and the conditions maintained for extraction of different phases for one gram of the sample are described below. The terminology used is the same as that used by Tessier et al., (1979).

I) **Exchangeable (T1 fraction):** 1g sample is extracted with 8 ml of unbuffered, 1.0M MgCl₂ (pH 7.0) with continuous agitation for one hour at 20 ± 2 °C.

II) **Carbonate (T2 fraction):** Residue from exchangeable fraction, is extracted with 8 ml of 1.0M NaOAc (adjusted to pH 5 with HOAc) with continuous agitation for 5 hours at 20 ± 2 °C.

III) **Fe-Mn Oxide bound (T3 fraction):** Residue from carbonate fraction, is extracted with 20 ml of 0.04M NH₂OH.HCl in 25% (v/v) HOAc at 96 °C with occasional agitation for 6 hours.

IV) **Organic bound (T4 fraction):** Residue from Fe-Mn oxide bound fraction is extracted with 3 ml of 0.02M HNO₃ and 5.0 ml of 30% H₂O₂ (adjusted to pH 2 with HNO₃). The mixture is heated to 85 °C for 2 hours, with occasional agitation. A second 3 ml aliquot of 30% H₂O₂ (pH 2 with HNO₃) is added and the mixture heated again for 3h with intermittent agitation and then 5 ml of 3.2M NH₄OAc in 20% HNO₃ (v/v) is added with continuous agitation for 0.5 h at 25 °C. The final volume is made to 20 ml.
V) Residual (TS fraction): Residue from organic fraction is digested using the HF - HNO₃ - HClO₄ dissolution procedure as used for the total metal extraction.

All the extractions were carried out in duplicates. The extractions except the residual fraction were carried out in 100ml polypropylene centrifugation tubes with tight lids. When continuous agitation was required, samples were shaken lengthwise on an end-to-end mechanical shaker. Heating of samples except the residual fraction was accomplished by using a water bath.

Cadmium, lead, zinc and manganese in digests and extracts, were determined by flame atomic absorption spectrometry (FAAS) using a Shimadzu AA-6800 and Philips PU 9200X system with air/acetylene flame. Calibrants were prepared from 1000 µg/l spectrosol standard solutions, from Merck. These were made up in each extractant and acidified to 1% with nitric acid.