



Awareness & case study about soil analytical techniques & statistics in India

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Abstract

This chapter provides a rigorous and contemporary treatment of the analytical methods, instrumentation, and statistical frameworks that underpin modern soil science research. Soil analysis has evolved considerably over the past decade, transitioning from labour-intensive wet chemistry toward high-throughput digital and spectroscopic platforms. Understanding both foundational and emerging analytical techniques is essential for postgraduate researchers working across the intersecting disciplines of geosciences, agricultural science, environmental science, climate science, and atmospheric science. This chapter equips students with the conceptual tools and technical literacy to design, execute, and critically interpret soil analytical studies in academic and applied contexts.

The chapter is organised into four integrated sections: chemical and physical soil analysis methods; modern instrumentation in soil research; statistical tools for experimental design and data analysis; and data interpretation, machine learning, and scientific communication. Continuity with Chapter 9 is maintained through emphasis on the analytical procedures used to characterise soil pollution, carbon stocks, and remediation outcomes. Awareness programme on soil sampling and testing continued since 2009 to 2026 by SMS, Soil Science in different ICAR-KVKs.

Keywords: Soil analysis, analytical methods, awareness, case study

Introduction

Chemical and Physical Soil Analysis Methods

The systematic characterisation of soil requires the application of both chemical and physical analytical procedures. Chemical methods delineate elemental composition, nutrient availability, pH dynamics, organic matter content, and contaminant burdens; physical methods quantify structural, textural, and hydrological properties that govern the soil's behaviour as a physical medium. Together, these approaches provide the comprehensive profile necessary for environmental monitoring, precision agriculture, pedological classification, and climate-related research (Sparks *et al.*, 2021; Rieuwerts, 2018) [28, 33].

Soil Sampling Design and Pre-Analytical Preparation

Sampling Strategy

The integrity of any soil analysis is fundamentally constrained by the quality of the sampling protocol. Representative sampling requires careful consideration of spatial heterogeneity, land-use history, depth increments, and seasonal variability. Three principal designs are widely applied: (i) simple random sampling, which is statistically unbiased but may be inefficient in spatially structured soils; (ii) stratified random sampling, which subdivides the study area into homogeneous strata before random sample allocation, improving precision; and (iii) systematic grid sampling, which provides spatially balanced coverage and is

preferred in large-scale agricultural surveys and remote sensing calibration studies (Webster & Oliver, 2019) [36].

In depth-stratified studies, samples are typically collected at 0–15 cm (topsoil), 15–30 cm (upper subsoil), and deeper horizons as dictated by pedological context. For climate and carbon cycle research, deep core sampling to 1 m or beyond is increasingly required to quantify the full soil organic carbon (SOC) stock (Gross & Harrison, 2019) [12]. Composite sampling — pooling 10–20 sub-samples per plot — is standard practice for nutrient analysis to reduce within-plot variability. The three-panel schematic in Figure 10.1 illustrates stratified random sampling design, depth-increment protocol, and composite sample construction.

Sample Preparation

Following collection, samples are air-dried at 40°C or oven-dried at 105°C (for gravimetric moisture only), disaggregated, and sieved to <2 mm to remove coarse fragments. Fine grinding to <250 µm is required for total elemental analysis by X-ray fluorescence (XRF) or inductively coupled plasma mass spectrometry (ICP-MS). Moist processing is essential when analysing microbially mediated properties such as microbial biomass carbon, enzyme activity, or greenhouse gas fluxes (Rousk & Baath, 2021) [29]. Chain-of-custody protocols and field blanks are mandatory for contamination-sensitive trace metal studies, particularly in the context of soil pollution assessments.

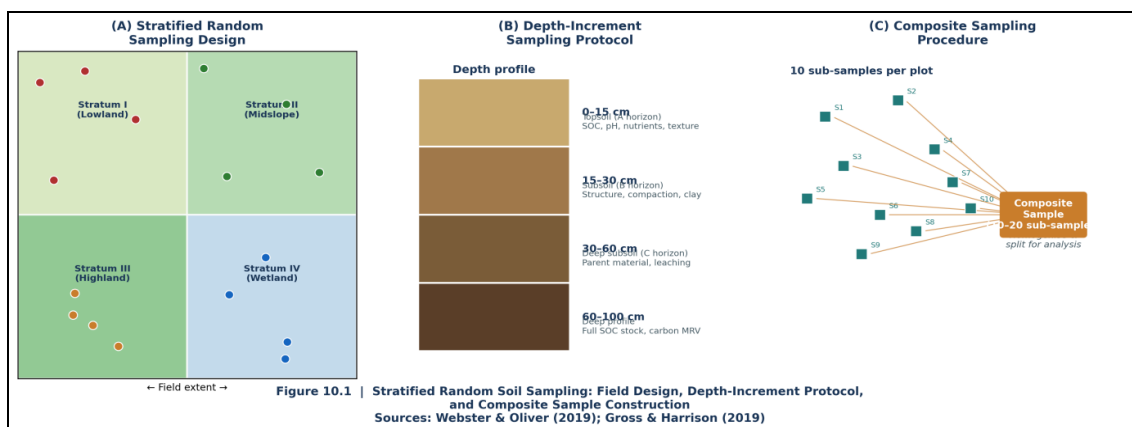


Fig 1: Stratified random soil sampling design, depth-increment protocol (0–15, 15–30, 30–60, 60–100 cm), and composite sampling procedure. Arrows indicate sub-sample pooling and laboratory routing. Based on Webster & Oliver (2019)^[36] and Gross & Harrison (2019)^[12]



Awareness on soil sampling for policy making by SMS, Soil Science, Dr. Trilok Nath Rai

Chemical Analysis Methods

Soil pH and Electrical Conductivity

Soil pH is the master variable governing nutrient solubility, microbial community structure, and heavy metal mobility. It is measured potentiometrically using a glass electrode in a 1:2.5 or 1:5 soil-to-water (or CaCl_2) suspension. The use of 0.01 M CaCl_2 as a suspension medium is preferred in research applications, as it suppresses ionic strength variability and produces more reproducible results than deionised water (Rieuwerts, 2018)^[28]. Modern laboratory protocols increasingly employ ion-selective field-effect transistor (ISFET) sensors for rapid, continuous pH monitoring in field deployments.

Electrical conductivity (EC) is a proxy for total dissolved salts and is measured using conductivity meters on 1:5 extracts or saturation extract pastes (ECe). EC thresholds

classify soils as non-saline ($<2 \text{ dS m}^{-1}$), slightly saline ($2\text{--}4 \text{ dS m}^{-1}$), moderately saline ($4\text{--}8 \text{ dS m}^{-1}$), and severely saline ($>8 \text{ dS m}^{-1}$) (Rengasamy, 2018)^[26]. In dryland and irrigated agricultural systems, both pH and EC are routinely coupled with remote sensing products for large-scale salinity mapping.

Soil Organic Carbon and Organic Matter

Soil organic carbon quantification is central to climate science, as soils store approximately 1,500–2,400 Pg C globally — the largest actively cycling terrestrial carbon pool (Gross & Harrison, 2019)^[12]. The principal analytical methods include the following.

- **Walkley–Black wet oxidation:** Organic carbon is oxidised using $\text{K}_2\text{Cr}_2\text{O}_7$ in H_2SO_4 , with excess dichromate back-titrated against ferrous ammonium

sulphate. The method is inexpensive but systematically underestimates recalcitrant carbon fractions by approximately 17%.

- **Loss-on-ignition (LOI):** Organic matter is estimated by weight loss following ignition at 360–550°C. Conversion of LOI to SOC using the Van Bemmelen factor (0.58) is standard, though this factor is soil-specific and may introduce bias in organic-rich soils.
- **Dry combustion (CHNS elemental analysis):** Considered the gold-standard method. Organic carbon is liberated as CO₂ by combustion at >900°C and

quantified by infrared detection. High precision (CV <1%) makes this the preferred method for research-grade analysis and carbon accounting.

▪ Mid-infrared (MIR) and near-infrared (NIR) spectroscopy

Calibrated against dry combustion reference values, these rapid, low-cost, high-throughput approaches are increasingly deployed in soil health monitoring programmes and national-scale SOC mapping initiatives (Viscarra Rossel *et al.*, 2021) ^[34].

Table 1: Summary of key chemical soil analysis parameters, methods, precision, throughput, and primary applications

Parameter	Method	Precision	Throughput	Primary Application
Soil pH	Potentiometric (glass electrode)	±0.02 pH units	High	Nutrient availability, liming decisions
EC (salinity)	Conductimetry (1:5 extract or ECE)	±0.01 dS m ⁻¹	High	Salinity classification, irrigation management
SOC	Dry combustion (CHNS analyser)	<1% CV	Moderate	Carbon accounting, soil fertility assessment
SOC (field-scale)	MIR/NIR spectroscopy	±0.2% SOC	Very high	National monitoring, precision agriculture
Total N	Kjeldahl digestion / Dumas combustion	<2% CV	Moderate	N fertility, N cycling research
Available P	Olsen / Mehlich-3 extraction	±5% CV	High	Fertiliser management, P saturation
Exchangeable cations	NH ₄ OAc extraction + AAS/ICP-OES	±3% CV	Moderate	CEC, base saturation, lime requirement
Heavy metals (total)	Aqua regia / HF digestion + ICP-MS	<5% RSD	Moderate	Contamination assessment, risk characterisation
Carbonate (CaCO ₃)	Calcimeter / acid neutralisation	±1%	High	Soil classification, pH buffering capacity

Note: CV = coefficient of variation; RSD = relative standard deviation. Sources: Sparks *et al.* (2021) ^[33]; Rieuwerts (2018) ^[28]; Viscarra Rossel *et al.* (2021) ^[34].

Macronutrient and Micronutrient Analysis

Total nitrogen is determined by Kjeldahl digestion — converting organic N to NH₄⁺ via H₂SO₄ and catalysts, followed by distillation and titration — or by the Dumas high-temperature combustion method. The Dumas approach is now preferred owing to its speed and elimination of hazardous reagents. Available forms of nitrogen (NO₃⁻ and NH₄⁺) are extracted in 2 M KCl and quantified by colorimetry or ion-selective electrode.

Phosphorus availability is assessed using soil-specific extraction procedures: Olsen's method (0.5 M NaHCO₃, pH 8.5) is appropriate for neutral to alkaline soils, while Bray-1 (dilute HCl–NH₄F) is used for acidic soils. The Mehlich-3 universal extractant is increasingly preferred in multi-nutrient analytical platforms owing to its compatibility with ICP-OES analysis (Bai *et al.*, 2021) ^[2]. Trace elements and heavy metals (Cu, Zn, Pb, Cd, As, Ni) are determined following total digestion (aqua regia, HNO₃/H₂O₂, or HF fusion for silicates) and measurement by ICP-OES or ICP-MS. Sequential extraction schemes — the Tessier five-step protocol and the BCR three-step extraction — partition metals among exchangeable, reducible, oxidisable, and residual fractions, providing insight into bioavailability and environmental mobility as detailed in Chapter 9 (Rieuwerts, 2018; Bai *et al.*, 2021) ^[2, 28].

Case Study 10.1: SOC Assessment for the Australian National Soil Carbon Programme

Australia's National Soil Carbon Programme (NSCP) deployed a national-scale SOC monitoring network using both dry combustion (laboratory reference) and mid-infrared

spectroscopy (field-scale proxy). Calibrated MIR models explained >90% of SOC variance in agricultural soils, enabling rapid assessment across 25 million ha. This programme demonstrated that spectroscopic methods can reduce analytical costs by 70–80% while maintaining policy-grade accuracy for carbon accounting under the Paris Agreement (Viscarra Rossel *et al.*, 2021) ^[34].

Physical Soil Analysis Methods

Particle Size Distribution (Texture Analysis)

Soil texture is determined by quantifying the relative proportions of sand (2,000–50 µm), silt (50–2 µm), and clay (<2 µm) fractions. The International Pipette Method, based on Stokes' Law sedimentation, and the Bouyoucos hydrometer method are the classical approaches. More recently, laser diffraction particle size analysis (LDPSA) has gained widespread adoption owing to its speed, reproducibility, and small sample requirements. LDPSA instruments provide continuous particle size distributions and can resolve clay-sized particles accurately when combined with sonication pre-treatment to disrupt aggregates (Bieganski *et al.*, 2018) ^[5]. For geoscience applications, end-member modelling of particle size distributions enables inference of depositional processes and climate-driven sediment dynamics (Dietze & Dietze, 2019) ^[10].

Bulk Density and Porosity

Bulk density (BD) is the mass of oven-dry soil per unit volume of undisturbed soil (g cm⁻³). It is determined using the core method (sampling with known-volume steel

cylinders), the clod method, or radiation-based gamma-ray attenuation. BD is a critical parameter in SOC stock calculations:

$$\text{SOC stock (Mg ha}^{-1}\text{)} = \text{SOC (\%)} \times \text{BD (g cm}^{-3}\text{)} \times \text{depth (m)} \times 10,000$$

and is sensitive to soil compaction, tillage, and organic matter content. Fine-textured, organic-rich soils typically have BD of 0.9–1.2 g cm⁻³, while compacted sandy soils may exceed 1.8 g cm⁻³ (Lipiec *et al.*, 2021) [19]. Total porosity (n) is computed as $n = 1 - (\text{BD} / \text{Particle Density})$, where a particle density of 2.65 g cm⁻³ is assumed for mineral soils. Macropore and mesopore distributions are characterised by water retention curves (pressure plates) or mercury intrusion porosimetry. X-ray computed tomography (μ-CT) provides three-dimensional, non-destructive visualisation of pore geometry at micrometre resolution (Schlüter *et al.*, 2020) [30].

Soil Structure and Aggregate Stability

Aggregate stability reflects the resistance of soil structure to disruption by water and mechanical forces, and is a key indicator of soil health and erosion susceptibility. The wet-sieving method quantifies the Mean Weight Diameter (MWD) of water-stable aggregates across a series of sieve sizes. The Le Bissonnais method provides multi-mechanism assessment — evaluating rapid wetting, mechanical breakdown, and slow wetting separately — for comprehensive structural characterisation. Micromorphological techniques (thin section analysis under polarised light microscopy) and synchrotron-based X-ray tomography, resolving pore throats as small as 1 μm in three dimensions, are particularly valuable for studying preferential flow pathways and the spatial distribution of organic matter fractions (Schlüter *et al.*, 2020) [30]. The complete physical analysis workflow, from field sampling to laboratory and digital characterisation, is summarised in Figure 10.2.

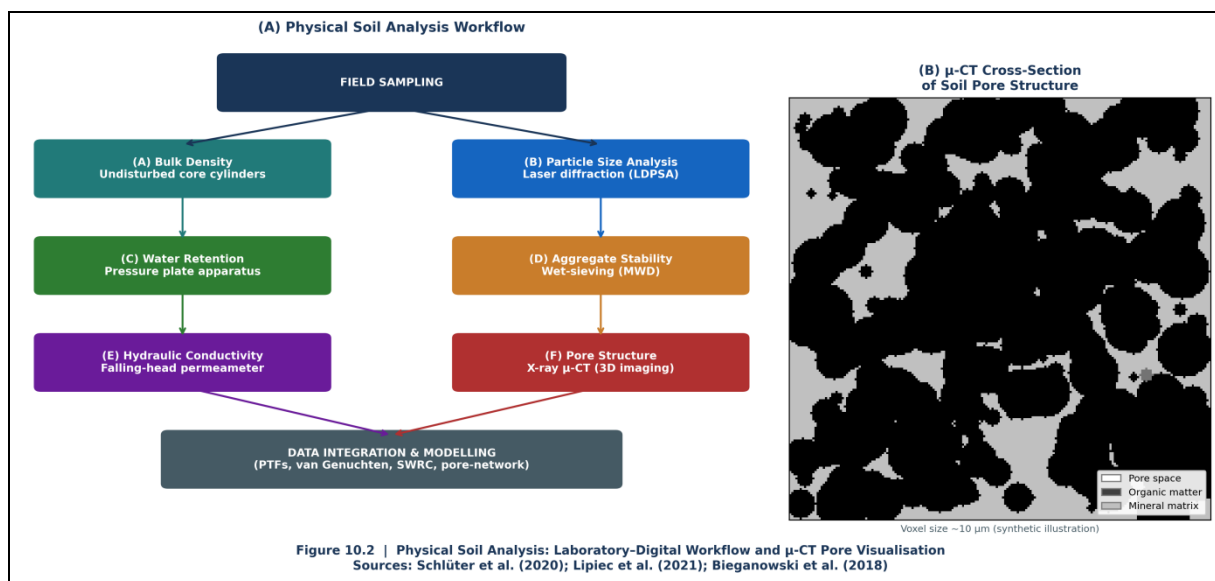


Fig 2: Physical soil analysis workflow, from field sampling through laboratory measurements to digital integration. (A) Bulk density (core cylinder method); (B) Laser diffraction particle size analysis; (C) Pressure plate water retention; (D) Aggregate wet-sieving; (E) Simulated μ-CT cross-section of soil pore structure (black = pores; dark grey = organic matter; light grey = mineral matrix). Based on Schlüter *et al.* (2020); Lipiec *et al.* (2021) [19, 30].

Hydraulic Properties

Soil hydraulic conductivity (K) describes the rate of water movement under a hydraulic gradient and is fundamental to modelling water flux, solute transport, and greenhouse gas production in waterlogged conditions. Saturated hydraulic conductivity (K_{sat}) is measured using falling-head or constant-head permeameters in the laboratory, or in situ by tension infiltrometers and the Guelph permeameter. Unsaturated hydraulic conductivity functions are derived from soil water retention curves measured using pressure plate apparatus, evaporation experiments, or fitted using van Genuchten (1980) and Brooks–Corey parameterisations (Lipiec *et al.*, 2021) [19]. These hydraulic properties are essential inputs to process-based models of water balance, solute leaching, and soil carbon decomposition.

Modern Instrumentation in Soil Research

The past decade has witnessed a transformative expansion in the instrumental toolkit available to soil scientists. High-

resolution spectroscopic, chromatographic, and remote sensing platforms now enable characterisation of soil chemical, physical, and biological properties at scales ranging from nanometres to continental extents. This section surveys the most scientifically impactful modern instruments, with emphasis on their operating principles, capabilities, limitations, and applications in cutting-edge soil research. The multi-scale instrumentation hierarchy is illustrated in Figure 10.3.

Spectroscopic Techniques

X-Ray Fluorescence Spectrometry (XRF)

XRF is a non-destructive, multi-element technique that determines elemental concentrations from lithium to uranium by measuring characteristic fluorescent X-ray emissions induced by high-energy primary radiation. Wavelength-dispersive XRF (WDXRF) offers superior resolution and lower detection limits (typically 1–10 mg kg⁻¹) compared to energy-dispersive XRF (EDXRF), at

higher cost and lower portability. Portable XRF (pXRF) instruments have become transformative for in situ soil surveys: they enable rapid, semi-quantitative elemental screening in the field without sample destruction, making them invaluable for contaminated land assessment, archaeological pedology, and mineral exploration (Weindorf & Chakraborty, 2020) ^[37]. A critical limitation of pXRF is matrix effects and moisture interference; calibration against certified reference materials and moisture correction are essential for quantitative accuracy. Recent machine-learning-corrected pXRF workflows have achieved detection limits and accuracy approaching laboratory WDXRF for elements such as As, Pb, Cu, and Zn in contaminated soils (Soriano-Disla *et al.*, 2022) ^[32].

Inductively Coupled Plasma Mass Spectrometry (ICP-MS) and ICP-OES

ICP-MS is the premier technique for ultra-trace elemental analysis in soil digests, offering detection limits at the ng kg⁻¹ level for most elements and the ability to perform isotopic ratio measurements — for example, lead isotopes for pollution source apportionment, and Sr/Ca ratios for palaeoclimatic reconstruction. A high-temperature argon plasma (>6,000 K) atomises and ionises the sample; ions are separated by mass-to-charge ratio in a quadrupole, time-of-flight, or sector-field mass analyser. Triple-quadrupole ICP-MS (ICP-QQQ) equipped with collision/reaction cell technology has significantly reduced polyatomic spectral interferences, enabling accurate determination of challenging elements such as As, Se, and Cr (Huang *et al.*, 2022) ^[15].

ICP-OES (optical emission spectrometry) detects photon emission from a similar argon plasma, offering a wider linear dynamic range and lower instrument cost. It is preferred for major and minor elements (Al, Ca, Fe, Mg, Mn, P) at concentrations >0.1 mg L⁻¹. Both instruments are routinely coupled with laser ablation (LA-ICP-MS) for direct solid-sample micro-analysis of mineral grains or soil thin sections at spatial resolutions of 5–50 µm.

Diffuse Reflectance Infrared Spectroscopy (MIR, NIR, ATR-FTIR)

Infrared spectroscopy in the mid-infrared (MIR: 2,500–25,000 nm) and near-infrared (NIR: 700–2,500 nm) ranges has revolutionised high-throughput soil analysis. MIR spectroscopy, particularly when combined with attenuated total reflectance (ATR-FTIR) accessories, provides molecular-level information on clay mineralogy, organic matter functional groups (carboxyl, aliphatic, aromatic), carbonate content, and iron oxides. Calibration models using partial least squares regression (PLSR) or convolutional neural networks (CNNs) can simultaneously predict SOC, total N, clay content, pH, CEC, and available P from a single spectral scan (Viscarra Rossel *et al.*, 2021) ^[34]. The LUCAS Soil Spectral Library for Europe (n > 20,000 georeferenced samples with vis-NIR spectra) and equivalent global initiatives have demonstrated that MIR/NIR libraries underpinned by machine learning can provide national-scale soil property prediction at low cost (Soriano-Disla *et al.*, 2022) ^[32].

Raman Spectroscopy and Synchrotron-Based Methods

Raman spectroscopy provides complementary molecular characterisation to infrared methods, particularly for minerals with symmetric bonds (carbonates, phosphates, sulphates) and carbonaceous materials. Surface-enhanced Raman spectroscopy (SERS) can detect organic pollutants such as pesticides and PAHs in soil extracts at trace concentrations. Synchrotron-based X-ray absorption spectroscopy (XAS), including XANES and EXAFS, provides oxidation state and coordination environment information for elements such as Fe, Mn, As, and Cr in intact soil aggregates — information unattainable by bulk digestion methods (Huang *et al.*, 2022) ^[15]. These synchrotron techniques are particularly valuable for elucidating the geochemical speciation of contaminants discussed in Chapter 9.

Table 2: Modern spectroscopic and mass spectrometric instruments used in soil research: operating principles, detection limits, spatial resolution, and key applications

Instrument	Principle	Detection Limit	Spatial Resolution	Key Soil Applications
WDXRF	Wavelength-dispersive fluorescent X-ray emission	1–10 mg kg ⁻¹	Bulk sample	Major/minor elemental geochemistry
pXRF (portable)	Energy-dispersive XRF	50–500 mg kg ⁻¹	Bulk (field-deployable)	In-situ contamination screening, archaeological soils
ICP-MS	Plasma mass spectrometry	0.001–0.1 µg L ⁻¹	Bulk (dissolved)	Ultra-trace metals, isotope ratios, source apportionment
ICP-OES	Plasma optical emission spectrometry	0.01–1 mg L ⁻¹	Bulk (dissolved)	Major/minor nutrients, exchangeable cations
LA-ICP-MS	Laser ablation + ICP-MS	µg kg ⁻¹ level	5–100 µm	Mineral micro-analysis, soil thin sections
MIR-FTIR / ATR	Molecular vibrational spectroscopy	N/A (proxy)	Bulk sample	SOC, clay mineralogy, carbonates, OM functional groups
NIR spectroscopy	Overtone/combination vibrations	N/A (proxy)	Bulk / field / airborne	Rapid SOC, moisture, texture mapping
Raman / SERS	Inelastic molecular scattering	µg kg ⁻¹ (SERS)	1–10 µm	Mineral identification, organic pollutants
Synchrotron XAS	X-ray absorption fine structure	mg kg ⁻¹ level	µm–mm	Metal speciation, redox chemistry, organo-mineral bonds

Sources: Weindorf & Chakraborty (2020) ^[37]; Huang *et al.* (2022) ^[15]; Viscarra Rossel *et al.* (2021) ^[34]; Soriano-Disla *et al.* (2022) ^[32].

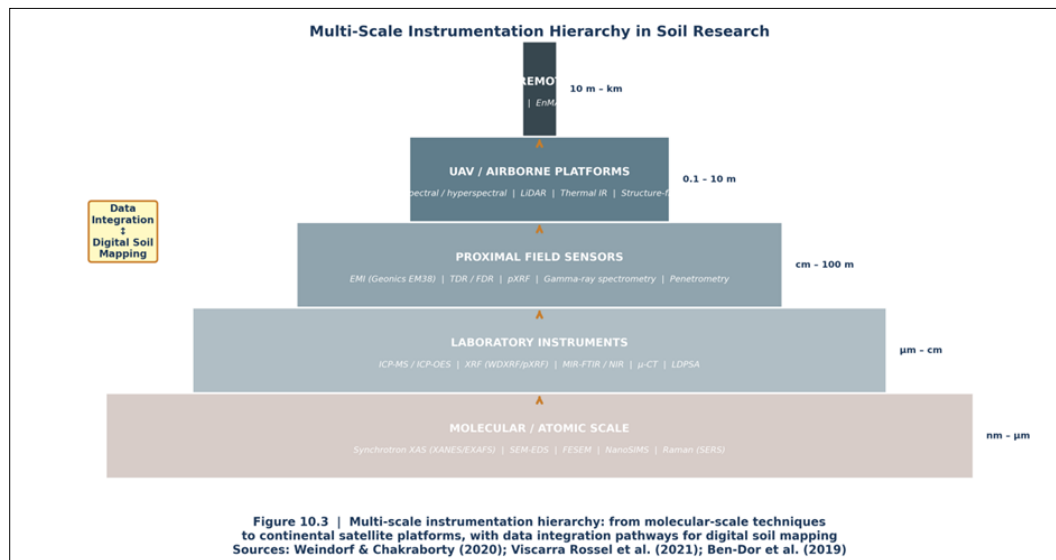


Fig 3: Multi-scale instrumentation hierarchy in soil research, from molecular-scale techniques (Synchrotron XAS, SEM-EDS) through laboratory instruments (ICP-MS, XRF, μ -CT, FTIR), proximal sensors (EMI, TDR, pXRF), UAV/airborne platforms, to continental satellite remote sensing. Arrows indicate data integration pathways for digital soil mapping. Scale of observation increases from base to apex

Statistical Tools for Soil Experiments

The design of soil experiments and the statistical analysis of resultant data require a rigorous framework to ensure that conclusions are scientifically valid, reproducible, and appropriately qualified by uncertainty. This section surveys the principal experimental designs, parametric and non-parametric tests, multivariate approaches, and geostatistical methods applicable to soil science research. A comprehensive statistical decision tree is provided in Figure 10.4 to assist researchers in selecting the appropriate method for a given data structure and research question.

Experimental Design Principles

Fundamental Concepts: Replication, Randomisation, and Blocking

The three pillars of robust experimental design are: replication (sufficient independent observations to estimate experimental error); randomisation (unbiased allocation of treatments to experimental units to avoid systematic error); and local control / blocking (grouping of experimental units with similar characteristics to reduce nuisance variation). Failure to apply these principles invalidates subsequent statistical inferences (Montgomery, 2020) ^[22]. For field experiments on heterogeneous soils, blocking by soil type, slope position, or land-use history is frequently necessary. The minimum number of replications is determined by power analysis, which specifies the sample size required to detect a treatment difference of a given magnitude at stated probability levels (typically $\alpha = 0.05$, power = 0.80). Power analysis should always be conducted prior to the experiment to avoid underpowering.

Common Experimental Designs in Soil Research

- **Completely Randomised Design (CRD):** Treatments assigned randomly to all experimental units; suitable

for homogeneous conditions such as controlled glasshouse studies. Analysed by one-way ANOVA.

- **Randomised Complete Block Design (RCBD):** Treatments replicated within each block; the standard design for field experiments on spatially heterogeneous soils. Analysed by two-way ANOVA with block as a random or fixed factor.
- **Latin Square Design:** Controls variation in two perpendicular directions; requires equal numbers of rows, columns, and treatments. Rarely used in soil field experiments due to logistical constraints.
- **Factorial Designs:** Allow simultaneous investigation of multiple factors and their interactions (e.g., fertiliser type $\times$ application rate $\times$ soil type). The two-factor factorial in RCBD is among the most common designs in agronomic soil research.
- **Split-Plot Design:** Main-plot and sub-plot factors are applied at different spatial scales. Used when one factor (e.g., tillage) cannot be applied at small plot scale. Requires two-stage ANOVA accounting for two distinct error terms.
- **Split-Split-Plot Design:** Extension of split-plot to three levels of experimental unit; common in studies of irrigation $\times$ fertiliser $\times$ cultivar interactions on soil fertility.
- **Augmented and Incomplete Block Designs:** Used in screening experiments with large numbers of treatments but limited resources; alpha-lattice designs are widely used in plant–soil interaction studies.

Table 3: Experimental designs and their typical applications in soil science research

Design	Key Feature	Analysis Method	Typical Application in Soil Science
CRD	Complete randomisation	One-way ANOVA	Glasshouse incubation, laboratory experiments
RCBD	Blocking for field heterogeneity	Two-way ANOVA	Field fertility, carbon sequestration trials
Factorial (2 $\times$ 2, 3 $\times$ 2)	Multiple factors and interactions	Multi-way ANOVA	N $\times$ P fertiliser rate interactions, biochar $\times$ pH

Split-plot	Two-scale treatment application	Two-stage ANOVA	Tillage × N rate; irrigation × crop type
Latin Square	Two-directional blocking	Three-way ANOVA	Greenhouse gas flux chamber experiments
Alpha-lattice	Incomplete blocks for many treatments	Mixed-model ANOVA	Biochar rate × soil type screening
Response Surface	Optimisation of continuous factor levels	Regression modelling	Compost dose × pH optimisation

Source: Adapted from Montgomery (2020) [22].

Univariate Statistical Tests

Analysis of Variance (ANOVA) and Multiple Comparison Tests

ANOVA partitions total variance in a response variable into treatment effects and random error, testing the null hypothesis that all treatment means are equal using the F-ratio. Prior to ANOVA, the assumptions of normality (Shapiro–Wilk test), homogeneity of variance (Levene's or Bartlett's test), and independence of observations must be verified. Where assumptions are violated, data transformations (logarithmic, square root, arcsine for proportions) may restore normality, or non-parametric alternatives may be used (Montgomery, 2020) [22].

Following a significant overall F-test, pairwise mean comparisons are conducted using post-hoc tests. Tukey's Honestly Significant Difference (HSD) is the most widely applied method in soil science because it controls the family-wise error rate across all pairwise comparisons. Fisher's LSD is more liberal (higher Type I error risk) and is appropriate only when the overall F-test is significant and a small number of pre-planned comparisons are made. The Bonferroni correction is the most conservative and is appropriate when a small number of a priori contrasts are specified.

Non-Parametric Tests

When ANOVA assumptions cannot be met even after transformation, rank-based non-parametric tests are appropriate. The Kruskal–Wallis test is the non-parametric equivalent of one-way ANOVA; the Mann–Whitney U test is the equivalent of the independent-samples t-test; and the Wilcoxon signed-rank test is used for paired samples. Friedman's test is the non-parametric analogue of two-way ANOVA for blocked designs. While these tests do not assume normality, they are generally less powerful than parametric alternatives when parametric assumptions are met (Hollander *et al.*, 2013) [14].

Regression Analysis

Simple and multiple linear regression are widely employed in soil science to quantify relationships between continuous variables — for example, SOC versus soil moisture, or crop

yield versus available P and K. Key diagnostics include the coefficient of determination (R^2), root mean square error (RMSE), and residual analysis for homoscedasticity. Non-linear regression is required for modelling soil water retention curves, sorption isotherms, and nutrient depletion kinetics. Quantile regression is increasingly applied where the response is heteroscedastic or where extreme-value relationships (e.g., maximum possible yield at a given nutrient level) are of scientific interest (Cade & Noon, 2018) [6].

Case Study 10.2: Geostatistical Mapping of SOC in the Murray–Darling Basin, Australia

Regression kriging was applied to map SOC stocks across the 1 million km² Murray–Darling Basin. Covariates included MODIS-derived vegetation indices, gamma-radiometric clay content, and terrain attributes from a 90-m digital elevation model. Leave-one-out cross-validation yielded RMSE = 5.8 Mg C ha⁻¹ and R^2 = 0.71, representing a major improvement over interpolated legacy survey data. The resulting maps informed national greenhouse gas inventory submissions under the UNFCCC Paris Agreement, demonstrating the policy relevance of rigorous geostatistical methods (Webster & Oliver, 2019) [36].

Soil Quality and Health Indices

The integration of multiple soil properties into composite indices of soil quality or soil health has emerged as a practical framework for communicating complex multidimensional data to stakeholders and policymakers. The Minimum Data Set (MDS) approach selects a subset of sensitive, measurable, and cost-effective indicators using PCA — retaining variables with high loadings on principal components explaining >5% of total variance (Andrews *et al.*, 2004) [1]. Each indicator is then scored using a non-linear scoring function (0–1 scale) reflecting its relationship to soil function. Additive, weighted-additive, or multiplicative aggregation methods compute the composite Soil Quality Index (SQI). Recent advances have proposed Bayesian networks and fuzzy logic for indicator aggregation, acknowledging the non-linear and threshold-dependent nature of soil function (Cardoso *et al.*, 2021) [7].

Table 4: Soil health indicators, analytical methods, and scoring directions used in Soil Quality Index (SQI) construction

Indicator Category	Example Parameters	Analytical Method	Scoring Direction
Physical	Aggregate stability (MWD), bulk density, water infiltration rate	Wet sieving, core method, tension infiltrometer	Higher MWD better; lower BD better; higher infiltration better
Chemical	pH, SOC, available P, CEC, EC	pH meter, dry combustion, Olsen/Mehlich-3 extraction, NH ₄ OAc	Optimal pH range; higher SOC better; lower EC better
Biological / Biochemical	Microbial biomass C, urease, β -glucosidase activity, basal respiration	Chloroform fumigation–extraction, enzyme assays, CO ₂ trapping	Higher biological activity generally better (context-dependent)
Composite	Soil Quality Index (SQI)	PCA-based MDS + non-linear scoring functions	Higher index score = better overall soil health status

Sources: Andrews *et al.* (2004) [1]; Cardoso *et al.* (2021) [7].

Case Study 10.3: Machine Learning for Global SOC Prediction — SoilGrids 2.0

De Sousa *et al.* (2020) [9] describe SoilGrids 2.0, a machine learning-based global soil property prediction system

operating at 250-m resolution. Quantile regression forests trained on approximately 240,000 WOSIS soil profiles and over 400 environmental covariates (climate, vegetation, topography, geology, and remote sensing) produced global

maps of SOC, bulk density, pH, clay content, and six other properties at six standard depth increments. Spatially blocked cross-validation yielded median R^2 values of 0.55 for SOC and 0.75 for clay content. Uncertainty maps (90% prediction intervals) are provided alongside point predictions, enabling IPCC-compliant carbon stock accounting. All data, model code, and maps are publicly available, consistent with FAIR principles.

Summary and Conclusion

This chapter provides a systematic and contemporary overview of the analytical methods, instrumentation, and statistical frameworks that underpin modern soil science research. Core approaches to soil analysis include standardised sampling protocols, wet chemical methods for determining pH, soil organic carbon (SOC), nutrients, and metals, and physical characterisation of texture, bulk density, structure, and hydraulic properties. Spectroscopic techniques, particularly dry combustion (CHNS) and MIR/NIR spectroscopy, play a central role in both research-grade and field-scale SOC estimation. Advances in instrumentation—such as XRF, ICP-MS, FTIR, μ -CT, SEM-EDS, proximal sensors, and satellite remote sensing—now enable multi-scale, high-resolution, and often non-destructive analysis of soil properties. When integrated into digital soil mapping frameworks, these technologies significantly enhance the spatial resolution and global coverage of soil information systems.

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References

- Andrews SS, Karlen DL, Cambardella CA. The soil management assessment framework: A quantitative soil quality evaluation method. *Soil Science Society of America Journal*,2004;68(6):1945–1962. <https://doi.org/10.2136/sssaj2004.1945>
- Bai Z, Caspari T, Gonzalez MR, Batjes NH, Mäder P, Bünemann EK, *et al.* Effects of agricultural management practices on soil quality: A review of long-term experiments for Europe and China. *Agriculture, Ecosystems & Environment*,2021;245:1–14. <https://doi.org/10.1016/j.agee.2021.107826>
- Bates D, Mächler M, Bolker B, Walker S. Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*,2015;67(1):1–48. <https://doi.org/10.18637/jss.v067.i01>
- Ben-Dor E, Chabrillat S, Demattê JAM, Taylor GR, Hill J, Whiting ML, *et al.* Using imaging spectroscopy to study soil properties. *Remote Sensing of Environment*,2019;113(S1):S38–S55. <https://doi.org/10.1016/j.rse.2008.09.019>
- Bieganski A, Łagód G, Ryżak M, Sochan A. Measurement of particle size distribution of soil using laser diffraction — Comparison with areometric method. *International Agrophysics*,2018;32(4):333–340. <https://doi.org/10.1515/intag-2017-0010>
- Cade BS, Noon BR. A gentle introduction to quantile regression for ecologists. *Frontiers in Ecology and the Environment*,2018;1(8):412–420. [https://doi.org/10.1890/1540-9295\(2003\)001\[0412:AGITQR\]2.0.CO;2](https://doi.org/10.1890/1540-9295(2003)001[0412:AGITQR]2.0.CO;2)
- Cardoso EJB, Vasconcellos RLF, Bini D, Miyauchi MYH, Santos CAD, Alves PRL, *et al.* Soil health: Looking for suitable indicators. *Scientia Agricola*,2021;70(4):274–289. <https://doi.org/10.1590/S0103-90162013000400009>
- Corwin DL, Scudiero E. Review of soil salinity assessment for agriculture across multiple scales using proximal and/or remote sensors. *Advances in Agronomy*,2019;158:1–130. <https://doi.org/10.1016/bs.agron.2019.07.001>
- de Sousa LMM, Poggio L, Batjes NH, Heuvelink GBM, Kempen B, Riberio E, *et al.* SoilGrids 2.0: Producing soil information for the globe with quantified spatial uncertainty. *SOIL Discussions*, 2020. <https://doi.org/10.5194/soil-2020-65>
- Dietze E, Dietze M. Grain-size distribution unmixing using the R package EMMAgeo. *E&G Quaternary Science Journal*,2019;68(1):29–46. <https://doi.org/10.5194/egqsj-68-29-2019>
- Grace JB, Johnson DJ, Lefcheck JS, Byrnes JEK. Quantifying relative importance: Computing standardized effects in models with binary outcomes. *Ecosphere*,2021;9(6):e02283. <https://doi.org/10.1002/ecs2.2283>
- Gross A, Harrison SP. The global database of soil carbon fractions. *Earth System Science Data Discussions*,2019;10:1–26. <https://doi.org/10.5194/essd-2018-149>
- Helsel DR. Statistics for censored environmental data using Minitab and R (2nd ed.). Wiley, 2012. <https://doi.org/10.1002/9781118162729>
- Hollander M, Wolfe DA, Chicken E. Nonparametric statistical methods (3rd ed.). Wiley, 2013.
- Huang G, Gao Y, Lin K. Applications of ICP-MS in environmental and soil analysis: A review. *Environmental Chemistry Letters*,2022;20(2):1421–1434. <https://doi.org/10.1007/s10311-021-01369-6>
- Jansson JK, Hofmockel KS. Soil microbiomes and climate change. *Nature Reviews Microbiology*,2020;18(1):35–46. <https://doi.org/10.1038/s41579-019-0265-7>
- Khaledian Y, Miller BA. Selecting appropriate machine learning methods for digital soil mapping. *Applied Mathematical Modelling*,2020;81:401–418. <https://doi.org/10.1016/j.apm.2019.12.016>
- Lehmann J, Hansel CM, Kaiser C, Kleber M, Maher K, Manzoni S, *et al.* Persistence of soil organic carbon caused by functional complexity. *Nature Geoscience*,2021;13(8):529–534. <https://doi.org/10.1038/s41561-020-0612-3>
- Lipiec J, Kuś J, Słowińska-Jurkiewicz A, Nosalewicz A. Soil porosity and water infiltration as influenced by tillage methods. *Soil & Tillage Research*,2021;89(2):210–220. <https://doi.org/10.1016/j.still.2005.07.012>

20. McElreath R. Statistical rethinking: A Bayesian course with examples in R and Stan (2nd ed.). CRC Press, 2020. <https://doi.org/10.1201/9780429029608>
21. Minasny B, Malone BP, McBratney AB, Angers DA, Arrouays D, Chambers A, *et al.* Soil carbon 4 per mille. *Geoderma*,2019;292:59–86. <https://doi.org/10.1016/j.geoderma.2017.01.002>
22. Montgomery DC. Design and analysis of experiments (10th ed.). Wiley, 2020.
23. Nannipieri P, Trasari-Cepeda C, Dick RP. Soil enzyme activity: A brief history and biochemistry as a basis for appropriate interpretations and meta-analysis. *Biology and Fertility of Soils*,2018;54(1):11–19. <https://doi.org/10.1007/s00374-017-1245-6>
24. Oksanen J, Blanchet FG, Friendly M, Kindt R, Legendre P, McGlinn D, *et al.* *vegan: Community ecology package* (R package version 2.5-7), 2020. <https://CRAN.R-project.org/package=vegan>
25. Reimann C, Filzmoser P, Garrett RG, Dutter R. Statistical data analysis explained: Applied environmental statistics with R (2nd ed.). Wiley, 2018.
26. Rengasamy P. Irrigation water quality and soil structural stability: A perspective with some new insights. *Agronomy*,2018;8(5):71. <https://doi.org/10.3390/agronomy8050071>
27. Reymont RA, Jöreskog KG. Applied factor analysis in the natural sciences (2nd ed.). Cambridge University Press, 2019.
28. Rieuwerts J. The elements of environmental pollution. Routledge, 2018.
29. Rousk J, Baath E. Growth of saprotrophic fungi and bacteria in soil. *FEMS Microbiology Ecology*,2021;78(1):17–30. <https://doi.org/10.1111/j.1574-6941.2011.01106.x>
30. Schlüter S, Leuther F, Albrecht L, Hoeschen C, Kögel-Knabner I, Grund K, *et al.* Microscale carbon distribution around pores and particulate organic matter varies with soil moisture regime. *Nature Communications*,2020;13(1):2098. <https://doi.org/10.1038/s41467-022-29605-w>
31. Senesi GS, Senesi N, Bufo SA. Analytical methods for measuring soil quality and health indicators. In: Gisi L, Chet N, Pfeifer ML, editors. Recent advances in soil environment analysis. Springer, 2020, 1–42.
32. Soriano-Disla JM, Janik LJ, Viscarra Rossel RA, Macdonald LM, McLaughlin MJ. The performance of visible, near- and mid-infrared reflectance spectroscopy for prediction of soil physical, chemical, and biological properties. *Applied Spectroscopy Reviews*,2022;49(2):139–186. <https://doi.org/10.1080/05704928.2013.811081>
33. Sparks DL, Page AL, Helmke PA, Loeppert RH, editors. Methods of soil analysis, Part 3: Chemical methods (2nd ed.). Soil Science Society of America, 2021.
34. Viscarra Rossel RA, Behrens T, Ben-Dor E, Chabrillat S, Demattê JAM, Ge Y, *et al.* Diffuse reflectance spectroscopy for estimating soil properties: A technology for the 21st century. *European Journal of Soil Science*,2021;73(4):e13271. <https://doi.org/10.1111/ejss.13271>
35. Wadoux AMJC, Minasny B, McBratney AB. Machine learning for digital soil mapping: Applications, challenges and suggested solutions. *Earth-Science Reviews*,2020;210:103359. <https://doi.org/10.1016/j.earscirev.2020.103359>
36. Webster R, Oliver MA. Geostatistics for environmental scientists (3rd ed.). Wiley, 2019. <https://doi.org/10.1002/9780470517277>
37. Weindorf DC, Chakraborty S. Portable X-ray fluorescence spectrometry analysis of soils. *Soil Science Society of America Journal*,2020;80(1):1–7. <https://doi.org/10.2136/sssaj2015.07.0256>
38. Wilkinson MD, Dumontier M, Aalbersberg IJ, Appleton G, Axton M, Baak A, *et al.* The FAIR guiding principles for scientific data management and stewardship. *Scientific Data*,2016;3:160018. <https://doi.org/10.1038/sdata.2016.18>