## **UNIVERSITY OF MUMBAI**

Syllabus for the M.Sc. Sem. I & II

Program: M.Sc.

**Course: Life Sciences** 

(Credit Based Semester and Grading System with effect from the academic year 2012–2013)

#### Revised Syllabus of M.Sc. Semester I and II Life Sciences

#### **SEMESTER I (60 lectures: Credits 4)**

# PSLSC101: Biodiversity, Evolution, Environmental Biology and Environmental Technology

#### **UNIT I:** Diversity and Systematics (15 lectures)

Changing perspectives in understanding diversity from morphology / anatomy / physiology / biochemistry to immunology, genetics, genomics and proteomics, methods of identification of biodiversity. Molecular systematics.

#### **UNIT II:** Evolution (15lectures)

- 1. Origin of Life: Classical experiments current concepts, evolution of biological macromolecules.
- 2. Natural Selection: Darwinism, its critical review and modern theory of evolution
- 3. Human evolution
- 4. Genetic basis of evolution

### **UNIT III:** Environmental Biology (15lectures)

- 1. Ecosystems: Basics features / types, energy flow in ecosystems, ecological productivity ecosystem modelling and systems approach to resource management and conservation.
- Renewable energy and biofuels.
   Environmental health: Environmental stress and adaptations, Effect of pollutants on living. Systems, their interactions at cellular and molecular levels molecular epidemiology, mutagenecity, genotoxicity, carcinogenicity, human disorders related to environmental pollution, biomonitoring indicators, bioremediation of pollution.
- 4. Toxicology: Basic principles of toxicology including LD50and ED50, management of acute intoxication, natural detoxification biochemical and genetic mechanisms.

### **UNIT IV:** Environmental Technology (15lectures)

- Sources, generation, classification & composition of solid wastes.
   Solid waste management methods Sanitary land filling, Recycling,
   Composting, Vermi composting, Incineration, energy recovery from organic waste.
- 2. Solid Waste Management Plan, Waste minimization technologies, Hazardous Waste Management, Sources & Classification, physicochemical properties, Hazardous Waste Control & Treatment.
- 3. Sun as a source of energy, solar radiations and its spectral characteristics, fossil fuels-classification, composition, physicochemical characteristics and energy content of coal, petroleum and Natural gas.
- 4. Principles of generation of hydroelectric power, tidal power, thermal energy conversion, wind, geo thermal energy, solar collectors, photovoltaic, solar ponds, oceans.

#### PSLSC102: Cell-, Micro- & Molecular- Biology

## **UNIT I:** Cell biology and Immunology (15lectures)

- 1. Cell as a macromolecular assembly, cellular compartmentalization and organelle architecture.
- 2. Nucleosome, chromatin and chromosome structure, nucleic, acidprotein interactions: histones and non-histones, topoisomerases, helicases and DNA-binding proteins.
- 3. Basic & cellular immunology: Types of immunity & immune response, lymphoid organism structure and classes antibodies, antigen and antibody reactions, complement system, histocompatubility antigens (MHC) and their role in antigen presentation & cytotoxicity, cell adhesion molecules & their role in immunity.

### **UNIT II:** Microbiology (15lectures)

1. Bacteriology - Morphology and ultra structure of bacteria - morphological types - cell walls of archaebacteria, Gram negative

- and Gram positive eubacteria, Eukaryotes, L- forms. Cell wall synthesis, antigenic properties, Capsule types, composition and function, Cell membranes structure, composition and properties.
- 2. Virology Discovery, nomenclature, classification and general characters of viruses, Distinctive properties of viruses, Morphology and ultra structure, capsids and their arrangements, types of envelopes and their composition. Viral genome, their types and structures, Virus related agents- Viroids and prions.
- 3. Mycology: Classification and general features of fungi. Life cycle of *Penicillin, Saccharomyces* and *Fusarium*. Structure of fungal cells and growth Hyphae and non- motile unicells, motile cells, effect of environment on growth, prevention of fungal growth.
- 4. Phycology: Distribution of algae, Classification of algae, Algal nutrition, reproduction, green algae, diatoms, euglenoids, brown Rhodophyta, Microalgae.

#### **UNIT III:** Molecular Biology (15lectures)

- 1. Basics of gene structure, split genes, introns/exons and overlapping genes.
- 2. Eukaryotic genome complexity: C value paradox, Cot analysis and estimation of coding potential repetitive / satellite DNA, Gene clusters, pseudogenes, global view of genome organization (comparative genomics0 in prokaryotes and eukaryotes.)
- 3. DNA replication and its regulation, telomere replication.
- 4. DNA mutagenesis, repair and recombination (homologous, sitespecific and illegitimate), transposons and retotrasposons.
- 5. Functional organization of genes in eukaryotes: promoters, operators, enhancers, control elements, Open Reading Frame (ORF), operons, terminator regions, genes for mRNA, tRNA & rRNA and their independent RNA polymerases.
- 6. Global view of gene expression and its regulation at various levels, gene imprinting.
- 7. Membrane receptor interactions: G-protein coupled receptors, tyrosine kinase receptors, ser-thr kinase receptors and cytokine receptors structural features of transmembrane receptors, hormone-receptor interaction.

8. Signal transduction: Two component signalling in prokaryotes, basic concepts in eukaryotic signalling role of phospoinositides, ion channels and second messenger, representative signalling pathways and their role in metabolism (eg. Peptide hormone), development (eg. Steroid hormone) neuronal transmission, cell cycle control & apoptosis.

#### **UNIT IV: Molecular Microbiology (15lectures)**

- 1. Functional organization of genes in prokaryotes
- 2. Prokaryotic genome constitution
- 3. Specialized cloning strategies Expression vectors, promoter probe vectors, vectors for library construction artificial chromosomes.
- 4. Rationale for the design of vectors for the over-expression of recombination protein, selection of suitable promoter sequences, ribosome binding sites, transcription terminator, fusion protein tags, purification tags, protease cleavage sites and enzymes, plasmid copy number and inducible expression system.

### **PSLSC103:** Measurements and Techniques in Biology

## UNIT I: Biostatistics-I (15lectures)

- Introduction, scope, applications and uses of statistics, collection and classification of data, census and sampling surveys, graphs and diagrams, central tendency and its measures: arithmetic mean, median, dispersion and its measures: range and its coefficient, variance and standard deviation, coefficient of variation.
- 2. Bivariate data, scatter diagram and interpretation, calculation and interpretation of Karl Pearson's correlation coefficient, equation of the lines of regression and properties of regression lines.
- 3. Probability, definition, addition and multiplicative laws (without proof). Random variable and its distribution, binomial probability distribution, examples and conditions, means and variance, Poisson probability distribution, examples and conditions, means and variance, continuous variable, normal distribution, use of normal probability table for finding probabilities.

- 4. Population parameters and sample statistics, sampling techniques: simple random sampling, stratified random sampling, systematic sampling, standard error
- 5. Estimation: point & interval, Estimators of population mean & proportion (without proof), confidence intervals for population mean & proportion.
- 6. Testing of hypothesis: Hypothesis and its types, errors and its types, levels of significance, one-tailed. and two-tailed tests, tests for single mean and single proportion, equality of the two population means and two population proportions.

# UNIT II: Principles and Practice of Physicochemical Techniques-I (15lectures)

- 1. pH, Buffers and calorimetry: principles and theory, pH meters.
- 2. Colorimetry & Spectroscopy: Basic principles, nature of electromagnetic radiation, Beer-Lambert laws, colorimetric methods & instruments, principles of spectroscopy, types of fluorescence spectra-absorbance, emission, and single and double beam spectrophotometers, spectra, densitometers, flame photometers, nuorimeters, circular dichorism & their applications.
- 3. Microscopy: Basic principles, instrumentation, sample preparation for optical, phase contrast, interference, polarisation, inverted, fluorescence, confocal & electron microscopes & their applications.
- 4. Microtomy: Principles & types, sample preparation & sectioning parameters.
- 5. Centrifugation: Principles & types simple & differential, ultracentrifugation preparative & analytical.
- 6. X-ray. Crystallography Nuclear Magnetic Resonance (NMR) spectra, Magnetic Resonance Imaging (MO, lasers in biology and medicine.

# UNIT III: Principles and Practice of Physicochemical Techniques-II (15lectures)

- 1. Nucleic acid hybridization: Basic principles of radioactive and non-radioactive hybridisation techniques, optimizing the temperature, ionic concentration and other critical parameters.
- 2. Blotting techniques: Southern, northern, western, south-western blotting techniques; choice of membranes and blotting conditions.

### **UNIT IV:** Biostatistics-II (15lectures)

- 1. Populations and samples
- 2. Data, graphical presentation of data frequency distribution
- 3. Sample means and standard deviations
- 4. Probability and Probability distributions
- 5. Normal distribution -z and t distributions
- 6. Binomial and Poisson distributions

#### PSLSC104: Neuroscience, Physiology and Development Biology

#### **UNIT I:** Neuroscience (15lectures)

- 1. History and development of Neuroscience
- 2. Neuro anatomy & physiology
- 3. Biological Clocks and Circadian Rhythm

## **UNIT II:** Animal Physiology (15lectures)

- 1. Endocrine system: Structure, function and regulation of endocrine organs with special reference to hypothalamus, pituitary, adrenal and thyroid glands.
- 2. Muscular system: Types of muscles & functions and significance of each type, special reference to cardiac muscles and cardiac anatomy & physiology

## **UNIT III** Plant Physiology (15lectures)

- Plant Life processes Respiration, transpiration, photosynthesis, nutrition
- 2. Plant Life processes Light control of plant development (phytomorphogenesis) and the role of Photoreceptors (red/blue/UV), plant homeostatic genes and their role in organogenesis.

#### **UNIT IV: Developmental Biology (15lectures)**

- 1. Reproduction in plants microsporogenesis, megasporogenesis, fertilisation and early embryo development.
- 2. Seed dormancy, germination, vegetative growth, flowering, fruiting, senescence and their regulation by phytohormones, auxins, gibberellins, cytokines, ethylene, abscissic acid, mechanisms of phytohormone action.

#### Semester I

## PSLSCP101: Ecology and Biochemistry (60hrs: Credits 2)

- 1. Waste Water Analysis BOD, COD, pH, Hardness of waste water
- 2. Field work Biodiversity documentation of the visited area- Flora & Fauna
- 3. Phytosociological Studies in an ecosystem
- 4. Preparation of solutions of different concentrations. Conductivity and pH measurements
- 5. Enzyme assays extraction and estimation of enzyme activity
- 6. Purification of enzyme by ammonium sulphate precipitation / gel filtration

# PSLSCP102: Genetics and Molecular Biology (60hrs: Credits 2)

- 1. Human Karyotyping
- 2. Genotyping and phenotyping
- 3. PCR technique, using standard 16srRNA eubacterial primers
- 4. PCR-based molecular markers- RAPDs, ISSR markers.
- 5 Purification of DNA from an Agarose Gel
- 6 Plasmid DNA isolation and DNA Quantitation
- 7 SDS-PAGE of proteins, staining by Coomassie Blue and molecular weight determination.

# PSLSCP103: Techniques in Biology (60hrs: Credits 2)

1. Bioinformatics Computer & Internet resources for biology, database, BLAST search.

- 2. Public domain protein and nucleotide sequence databases (SWISS-PROT, GenBank, EMBL), Protein Structure Database (PDB), Computer tools for sequence analysis: finding and retrieving sequences.
- 3. Special (departmental) option: Histochemical localisation of carbohydrates, lipids and nucleic acids from germinating seeds and nucleic acids (DNA only) from Animal Tissue (Fuelgen Technique).
- 4. Microscopy- Theoretical considerations and instrumentation of Light, Phase-contrast, Interference, Polarization and Fluorescence microscopes.
- 5. Centrifugation: Differential and density gradient centrifugation, Zonal and isopycnic separation, Preparative and analytical centrifugation.
- 4. Chromatography: Paper and Thin layer chromatography, Adsorption column chromatography, Ion-exchange chromatography, Gel exclusion chromatography, High performance liquid chromatography. Affinity chromatography and Gas chromatography.
- 6. Electrophoresis: Horizontal and vertical gel electrophoresis.

# PSLSCP104: Neuroscience, Physiology and Development Biology (60hrs: Credits 2)

- 1. Neuronal Network practical aspects of human brain
- 2. Human Heart- working model- practical study
- 3. EEG tracing and its relevance & significance to brain functioning
- 4. Estimation of ascorbic acid in ripe and unripe fruits
- 5. Assaying IAA oxidase activity in green and senescent leaves
- 6. Studies on induction of amylase activity by GA3 in germinating cereal grains

#### SEMESTER II

# PSLSC201: Biodiversity Evolution and Environmental Biology (60 lectures: Credits 4)

#### **UNIT I:** Diversity and Systematics (15lectures)

Salient features of the systems of plant, animal and microbial taxonomy from Haeckel to Whittaker to Carl Woese and beyond.

### **UNIT II:** Evolution (15lectures)

- Evolution of early living forms, genetic / genomic evolution, recent insights from comparative genomics.
- Species & Speciation
- Adaptation, levels of selection and "altruism"
- Mendelian genetics

## **UNIT III:** Environmental Biology (15lectures)

- 1. Environmental impact of Genetically Modified Organisms (GMOs), identification of G in the environment, their effects on the ecosystem, emergence of drug / herbicide / pest resistance and disease burden. Ethical, Legal and Social issues in modern biology.
- 2. Biodiversity: Rio convention, material transfer and benefit sharing, intellectual prop rights, bioprospecting, biodiversity identification and conservation, gene banks, role biodiversity in agriculture, animal husbandry, industrial microbiology, forestry etc.
- 3. Climate Change: International Panel for Climate Change (IPCC), ozone layer deplete. Ozone depleting substances and ozone-friendly technologies, effect of agriculture / ani mal husbandry on climate change and global warming and vice versa.
- 4. Bioethics: Issues / Legislation, animals' rights, ethical issues in modern biology / biotechnology.

5. Biosafety: Handling, storage and disposal of hazardous biological, safely and environmental regulations of GMOs, laboratory safely and good laboratory practices (GLI).

#### **UNIT IV:** Environmental Technology (15lectures)

- 1. Hospital Waste Management, Hazardous Waste Management & Handling rules, 1989 & 2000 (amendments).
- 2. Disaster Management, Fly ash generation & utilization, Primary, secondary & tertiary & advance treatment of various effluents.
- 3. Nuclear energy- fission and fusion, bio energy -energy from biomass and biogas, anaerobic digestion, energy use patterns in different parts of the world. Impacts of large scale exploitation of solar, wind, hydro and ocean energy.
- 4. Mineral resources and reserves, ocean ore and recycling of resources, Environmental impact of exploitation, processing and smelting of Mineral, oceans as need areas for exploitation of Mineral resources.

### PSLSC 202: Cell, Micro & Molecular Biology

## **UNIT I:** Cell biology and Immunology (15lectures)

- 1. Cell-division, cell cycle and its regulation, role of cycling, CDKs and oncogenes.
- 2. Cell of the immune system, origin, maturation and differentiation of T cells & B cells, their subsets and functions, cytokines and their receptors-types, functions & disorders.
- 3. Molecular immunology: Gene rearrangements in Ig & TCR gene diversity, class witch structure of B-cell and T-cell receptors.
- 4. Applied immunology: Engineered antibodies (monoclonal, bispecific, chimeric phage display, vaccination (live & attenuated vaccines, recombinant & naked DNA vaccines).

## **UNIT II:** Microbiology (15lectures)

- 1. Microbial Biochemistry –
- i) Monosaccharides and their relationship, structure of sugars, stereoisomerism and optical isomers of sugars. Reactions of aldehyde and

ketone groups, Ring structures and tautomeric forms, Mutarotation, Reaction of sugars to -OH groups. Important derivatives of monosaccharides, disaccharides and trisaccharides. Structure, identification and importance of mono, oligosaccharides. Structure, occurrence and biological importance of. structural polysaccharides *e.g.* blood group.

- ii) Definition and classification of lipids. Building blocks of lipids, fatty acids, glycerol, sphingosine. Fatty acidsdistribution in nature, classification, physico-chemical properties, separation, characterization and chemical properties. Saponification and iodine number, Properties and function of phospholipids. Lipoproteinsclassification, composition and their importance. Role of lipids in cellular architecture and functions.
- iii) Amino Acids- structure, classification and properties, Handerson and Hasselbach equation for ionization of amino acids, Chemical reactions of amino acids, Synthesis of peptide bonds. Primary, secondary, tertiary and quaternary structure of proteins, Ramchandran plot. Determination of amino acid sequence in proteins / polypeptides.
- iv) Enzymes as biocatalysts. Enzyme classification, Mechanism of enzyme action specificity, active site, activity unit and isozymes. Factors affecting enzyme efficiency, enzyme activators, coenzymes and cofactors. Enzyme kinetics Michaelis Menton equation for simple enzymes, determination of kinetic parameters, multi-step reactions and rate limiting steps. Enzyme inhibition- reversible, irreversible, competitive and noncompetitive. Allostersim- kinetic analysis of allosteric enzymes, Principles of allosteric regulation.
- v) Vitamines Discovery, role and chemistry of fat soluble vitamins A, D E and K. Water soluble vitamins Pantothenic acid, niacin, pyridoxine, biotin, riboflavin, cyanocobalamine, folic acid and ascorbic acid.

### **UNIT III:** Molecular Biology (15lectures)

- 1. Transcription: Components of transcriptional machinery in prokaryotes and eukaryotes initiation elongation, termination, RNA polymerase (s) and their interaction with +ve and –ve regulators, sigma factors.
- 2. Transcription regulation strategies in prokaryotes bacteriophage Lambda and eukaryotes: Operons (lactose, tryptophan), regulons and stimulons, catabolite repression, DNA methylation, tissue specific and developmental stage-specific expression of genes, transcription factors.

- 3. RNA processing & Post-transcriptional gene regulation: Capping, polyadenylation, splicing and splice and developmental stage-specific expression of genes, transcription factors.
- 4. Translation: apparatus and mechanism, genetic code, codon preferences, ribosome structure assembly, components of translation, initiation, elongation and termination, translation fidelity, antibiotics / inhibitors of translation, regulation of translation.

### **UNIT IV:** Molecular Microbiology (15lectures)

- 1. RNA processing & Post-transcriptional gene regulation: Capping, polyadenylation, splicing and splice and developmental stage-specific expression of genes, transcription factors.
- 2. Translation: apparatus and mechanism, genetic code, codon preferences, ribosome structure assembly, components of translation, initiation, elongation and termination, translation fidelity, antibiotics / inhibitors of translation, regulation of translation.
- 3. Core techniques and essential enzymes used in r-DNA technology, Restriction digestion, ligation and transformation. Cloning vectors-Plasmids, phages and cosmids. Cloning strategies Cloning and selection of individual genes, gene libraries cDNA and genomic libraries.

#### **PSLSC203: Measurements and Techniques in Biology**

## UNIT I: Biostatistics-I (15lectures)

- 1. Chi-square test for independent attributes in r X c table, special case of 2 X 2 tables.
- 2. Student's test for significance of correlation coefficient y for p = o (Small sample tests fishers Z transformation coefficient for getting y p = 0 in large samples, test (significance for y (p = 0).
- 3. Design of experiment: Principles and concepts of completely randomised design randomised block design and Latin square design.
- 4. Non-parametric tests: Distribution-free methods, sign test for method pairs, Wilcox test for unpaired data, Run test.

# UNIT II: Principles and Practice of Physicochemical Techniques-I (15lectures)

- 1. Cell separation and flow cytometry, magnetic beads, elutriator.
- 2. Chromatography: Principles methodology and applications of chromatography using paper , thin layer, column (gel filtrations, ion exchange, affinity) gas, HPCL, FPCL etc.
- 3. Electrophoresis: Principles and types of electrophoresis and their applications for proteins, nucleic acids, including gradient gel and pulse-field gel electrophoresis; gel matrices polyacrylamide, agarose *etc* critical parameters for optimum separation and resolution, two dimensional electrophoresis (IEF).
- 4. Radioisotope methods and tracer techniques in biology: Basic principles of radioactivity, properties & handling of radioisotopes in biology & medicine, radiation units, Geiger Muller & scintillation counters, autoradiography, radio nuclide imaging, CT scan.
- 5. Role of computers in Biology: Basic hardware & principles, multimedia, operating system, software applications, imaging and analysis in biology, internet, bioinformatics.

# UNIT III: Principles and Practice of Physicochemical Techniques-II (15lectures)

- 1. Blotting techniques: Southern, northern, western, south-western blotting techniques choice of membranes and blotting conditions.
- 2. Cytogenetic techniques: Banding, karyotyping, in-situ hybridization, image analysis.
- 3. Immunological methods: Antibody production (poly/monoclonal) ELISA, immunodiffusion, immunoelectrophoresis, radioimmunoassay, immunohistochemistry.
- 4. Basic principles and applications of plant and animal tissues culture.

## **UNIT IV:** Biostatistics- II (15lectures)

- 1. Experimental designs- completely randomised, randomised block and factorial experimental designs
- 2. Analysis of variance for different experimental designs, F distribution
- Correlation and regression, linear and non-linear regression, multiple regression

## PSLSC204: Neuroscience, Physiology and Development Biology

#### **UNIT I:** Neuroscience (15lectures)

- 1. Fundamentals of nerve function, synapses, neurotransmitters
- 2. Technological, conceptual, and cultural influences that have shaped understanding of brain and nervous system.
- 3. Various states of brain activities (Conscious, subconscious, unconscious)

### **UNIT II:** Animal Physiology (15lectures)

- 1. Reproductive system: Differentiation of sex, structure, function and cellular interactions in mammalian testis and ovary, mechanism of ovulation and fertilisation, early embryonic development, implantation and placentation.
- 2. Cardiovascular system: Structure and function of human heart, mechanical events of cardiac cycle, heart diseases and disorders, human vascular systems- arterial and venous blood flow.

### **UNIT III:** Plant Physiology (15lectures)

- 1. Seed dormancy, germination, vegetative growth, flowering, fruiting, senescence
  - and their regulation by phytohormones, auxins, gibberellins, cytokinins, ethylene,
  - abscissa acid-jasmonate and salicylate, mechanisms of phytohormone action.
- 2. Light control of plant development (phytomorphogenesis) and the role of photoreceptors (red/blue/UV), plant homeotic genes and their role in organogenesis.

## **UNIT IV:** Developmental Biology (15lectures)

- 1. Differentiation of sex, structure, function and cellular interactions in mammalian testis and ovary, mechanism of ovulation and fertilisation, early embryonic development, implantation and placentation.
- 2. Concept of totipotency, determination and induction, role of maternal genes in early embryonic development, homeotic genes, pattern formation and morphogenesis (*e g* limb).

#### **PRACTICALS**

#### Semester II

## PSLSCP201: Ecology and Biochemistry

(60hrs: Credits 2)

- 1. Faunal studies: Determination of minimum number of quadrates, Estimation of frequency, abundance, dominance, I.V.I. (Importance Value Index), richness
- 2. Comparison of stomata index and pollen fertility of the plants from polluted and clear areas
- 3. Practical Study of Natural Habitats in Urban Ecosystems
- 4. Estimation of sugar by DNSA method.
- 5. Separation of lipids by TLC.
- 6. Separation of amino acids by paper chromatography.

## PSLSCP202: Genetics and Molecular Biology (60hrs: Credits 2)

- 1. Restriction digestion of plasmid DNA, electrophoresis and molecular weight determination of DNA fragments.
- 2. Transformation of *E. coli* with plasmid, selection and confirmation of transformants.
- 3. Isolation of plant genomic DNA and quantification
- 4. Effect of temperature and alkali on absorbance of DNA hyperchromicity
- 5. Southern hybridization of available genome with probe and non-radioactive detection.
- 6. Transformation of yeast Saccharomyces cerevisiae

# PSLSCP203: Techniques in Biology (60hrs: Credits 2)

- 1. Frequency distribution, standard deviation and standard error of mean, Use of z, and t tests, Chi-square test
- 2. Analysis of variance, linear regression and correlation

- 3. Spectrophotometry: Colourimetry, Spectrophotometry.
- 4. Spectroscopy: Absorption and emission spectroscopy. Theory, instrumentation and applications of visible, ultraviolet and infra red spectroscopy.
- 5. Absorption spectra of BSA / chlorophyll / DNA and determination of absorption maxima
- 6. Conductivity and pH measurements

## PSLSCP204: Neuroscience, Physiology and Development Biology (60hrs: Credits 2)

- 1. Cognition and tests for cognitive functions with special reference to PVTs (Psychomotor Vigilance Tests)
- 2. Cultural & Social influence on human circadian rhythm- case studies
- 3. Effect of gender bias on sex ratio- survey/review of available data
- 4. Effect of growth promoters & inhibitors (one each) on plant growth
- 5. Estimation of soluble proteins in germinating and non-germinating seeds by Lowry / Bradford's method
- 6. Estimation of total amino acids in germinating and non germinating seeds