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ENT-609 **Agriculture & Environmental Pollution** **3(2-1)**

Syllabus For Mid & Final Term Examination

Environmental pollution is "the **contamination** of the physical and biological components of the earth/atmosphere system to such an extent that normal **environmental processes** are adversely **affected.**

Pollution is the introduction of contaminants into the natural environment that causes adverse change. Pollution can take the form of chemical substances or energy, such as noise, heat or light, heat, sound and radioactivity etc.

A ***pollutant*** is a substance or energy introduced into the environment that has undesired effects, or adversely affects the usefulness of a resource.

Environmental pollutants are permanent parts of the pollution process. They are the actual "*executing agents*" of environmental pollution. They come in ***gaseous***, ***solid*** or ***liquid*** form.

Characteristics of Environmental Pollutants

1. Pollutants don't recognize boundaries, i.e. they are trans-boundary.
2. Many of them can't be degraded by living organisms and therefore stay in the ecosphere for many years.
3. They destroy biota and habitat.

Types of Environmental Pollution

Generally, there are three types of environmental pollution.

1. Air pollution
2. Water pollution
3. Soil pollution (contamination)

Some of the most notable **air pollutants** are sulfur dioxide, nitrogen dioxide, carbon monoxide, ozone, volatile organic compounds (VOCs) and airborne particles, with radioactive pollutants probably among the most destructive ones (specifically when produced by nuclear explosions). **Water pollutants** include insecticides and herbicides, food processing waste, pollutants from livestock operations, volatile organic compounds (VOCs), heavy metals, chemical waste and others. Some **soil pollutants** are: hydrocarbons, solvents and heavy metals. So where does environmental pollution come from?

Sources of Environmental Pollution

Fossil Fuel Sources of Environmental Pollution (oil, gas and coal etc.)

Fossil fuels are among the **most** serious sources of environmental pollution. Power-generating plants and transport are probably the biggest sources of fossil fuel pollution. Common **sources** of fossil fuel pollution are:

Industry:

- Power-generating plants
- Petroleum refineries
- Petrochemical plants
- Production and distribution of fossil fuels
- Other manufacturing facilities

Transport:

- Road transport (motor vehicles)
- Shipping industry
- Aircraft

Fossil fuel combustion is also a major source of **carbon dioxide** (CO_2) emissions and perhaps the most important cause of global warming.

Other (Non-Fossil Fuel) Sources of Environmental Pollution

Among other pollution sources, **agriculture** (livestock farming) is the largest generator of ammonia emissions resulting in *air pollution*. Chemicals such as pesticides and fertilizers are also widely used in agriculture, which may lead water pollution and soil contamination as well.

Trading activities may be another source of environmental pollution. For example, it's been recently noted that packaging of products sold in supermarkets and other retail outlets is far too excessive and generates large quantities of solid waste that ends up in landfills leading to *soil contamination* and *air pollution*.

Residential sector is another significant source of pollution generating solid municipal waste that may end up in landfills or incinerators leading to soil contamination and air pollution.

ENVIRONMENTAL POLLUTION: Introduction, Causes & Types

What is Environmental Pollution?

- Environment Pollution is the addition of contaminants into the natural environment that causes detrimental effects to nature, natural resources and mankind.
- Any unnatural and negative changes in all the dimensions like chemical, physical and biological characteristics of any component of the ecosystem i.e. air, water or soil which can cause harmful effects on various forms of life and property is called environmental pollution.

What is a Pollutant?

- Any substance which causes harmful effects or uneasiness in the organisms, then that particular substance may be called as the pollutant.

The materials that cause pollution are of two types:

1. **Persistent pollutants:** Those pollutants which remain consistent in the environment for a long period of time without any change in its original form are called persistent pollutants**.** For example pesticides, nuclear wastes, and plastics etc.

2. **Non-persistent pollutants:** These pollutants are the opposite of persistent pollutant and break down in the simple form. If this process of breaking down is done by living organisms, then such pollutants are referred to as biodegradable pollutants. From another perspective, pollutants can be classified as follows:

1. **Primary Pollutants:** Primary pollutants are those which remain in the form in which they were added to the environment for ex. DDT, Plastic

2. **Secondary Pollutants**: Secondary pollutants are formed due to interaction of primary pollutants amongst themselves viz. PAN by the interaction of NOx & Hydrocarbons. According to their existence in nature:

1. **Quantitative Pollutants:** These substances are already present in the atmosphere but they become pollutant when their concentration level reaches to a particular level which is above a threshold limit.
2. **Qualitative Pollutants:** These are man-made pollutants eg. Fungicides, herbicides etc.

   According to origin:

   1. **Man-made Pollutants**
   2. **Natural Pollutants**

According to the nature of disposal:

1. **Biodegradable Pollutants**

2. **Non-biodegradable Pollutants**

According to the nature of disposal:

1. **Biodegradable Pollutants**

2. **Non-biodegradable Pollutants**

Types of pollution:

Air Pollution:

- Air pollution is the presence of one or more disadvantageous content in such quantity and for such duration, as it is catastrophic, or tend to be catastrophic, to human health and welfare, animal or plant life.
- It is the contaminants of air by the discharge of detrimental substances.
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Some of the air pollutants, their sources and effects:

Name of the pollutants	**Sources**	**Health effects**
Nitrogen oxides	Industries, vehicles and power plants	Problems in the lungs, respiratory systems and causes asthma and bronchitis.
Carbon monoxide	Emission and burning of fossil fuels	Severe headache, irritation to mucous membrane, unconsciousness and death.
Carbon dioxide	Burning of fossil fuels	Vision problem, severe headache and heart strain.
Suspended particulate matter	Vehicular emission and burning of fossil fuels.	Lung irritation reduces development of RBC and pulmonary malfunctioning.
Sulphur oxide	Industries and power plant	Irritation in eyes and throat, allergies, cough etc.
Smog	Industries and vehicular pollution	Respiratory and eye problems
Hydrocarbons	Burning of fossil fuels	Kidney problems, irritation in eyes, nose and throat, asthma, hypertension and carcinogenic effects on

		lungs.
Chlorofluorocarbons	Refrigerators, emission from jets	Depletion of ozone layer, global warming

• Other pollutants are cadmium, lead, mercury, silica, coal dust and particles and radioactive pollutants.

Control measures

• Policy measures

• Modification of industrial process and selection of suitable fuels and its utilization.

• Collection of pollutants and convert it into less toxic forms by different methods.

Government initiatives

• National air quality monitoring programme (NAMP)

• National ambient air quality standards (NAAQS).

Water Pollution:

• Addition of certain substances such as organic, inorganic, biological and radiological to the water, which degrades the water quality and makes it unhealthy for use.

• Water pollution is not only confined to surface water but also spread to groundwater, sea and ocean.

Sources

Point sources: These are directly pointed towards the water bodies from the source of origin

of pollution and are thus easy to regulate.

Non-point sources: These sources are related to many diffuse sources and are thus difficult

to regulate.

Some of the sources are:

• Industrial and community wastewater: Industries like mining, iron and steel, pharmaceuticals, food processing, soap and detergent and paper and pulp.

• Agricultural sources, thermal pollution (discharge of hot water by thermal power plants cause deficiency of dissolved oxygen in water) and underground water pollution.

• Marine pollution: river discharge, manmade pollution and oil spills etc.

Effects

• An excessive amount of mercury in water can cause Minamata disease in humans and dropsy in fishes; Lead in large amount can cause dyslexia, Cadmium poisoning causes Itai – Itai disease etc.

• Polluted water has less amount of Dissolved oxygen (DO) content which is important for sensitive organisms, thereby eliminates sensitive organisms.

• Excess of nitrate in drinking water is dangerous for infants and human health, excess fluoride cause neuromuscular disorder and teeth deformity, hardening of bones and painful joints.

• Biological magnification and eutrophication.

Control measures

• Usage of water should be minimized by changing the techniques involved.

• Recycling and treatment of water should be used to the maximum extent possible.

• The quantity of discharge of wastewater can be minimized.

• Excessive use of pesticides and fertilizers should be avoided.

• Organic farming and efficient use of animal residues as fertilizers.

Soil Pollution:

• Addition of unwanted substances to the soil which negatively affects physical, chemical and biological properties of soil and reduces its productivity is called soil pollution.

• The factors which disturb the biological balance of the soil and deteriorate the quality, texture and mineral content are called soil pollutants.

• Use of fertilizers, pesticides, insecticides, dumping of solid waste, deforestation and pollution due to urbanization and other anthropogenic substances causes soil pollution.

Sources

• Industrial waste: lead, cadmium, mercury, alkalies, organic substances and chemicals.

• Agricultural waste: fertilizers, pesticides, insecticides and manures.

• Discarded materials and radioactive elements and plastic bags.

Effects

• Agriculture: It reduces soil fertility and thus crop yields; increase soil erosion and

salinity.

• Ecological imbalance and imbalance in flora and fauna further increases.

• Problems in urban areas like clogging in drains, release of gases, foul smells and problems in wastewater management.

• Release of radioactive rays, biomagnification and pollutant gases cause health problems.

Control measures

• Afforestation, reforestation and use of organic farming.

• Solid waste management and reduction of waste from the construction area.

• Stop the use of plastic bags and use bags of degradable materials like paper and cloth.

• Biomedical waste should be collected and incinerated in incinerators.

Environmental Deterioration/Degradation

Environmental degradation/deterioration is the disintegration of the earth or environment through consumption/destruction of assets, for example, air, water and soil; the destruction of environments and the eradication of wildlife. It occurs when earth's natural resources are depleted and environment is compromised in the form of extinction of species, pollution in air, water and soil, and rapid growth in population.

Causes of Environmental Deterioration/Degradation:

1. **Land Disturbance:** A more basic cause of environmental degradation is land damage. Numerous weedy plant species, for example, garlic mustard, are both foreign and indigenous. A rupture in the environmental surroundings provides for them a chance to start growing and spreading which destroy the natural vegetation.
2. **Pollution:** Pollution, in whatever form, whether it is air, water, land or noise is harmful for the environment. Air pollution pollutes the air that we breathe which causes health issues. Water pollution degrades the quality of water that we use for drinking purposes. Land pollution results in degradation of earth's surface as a result of human activities. Noise pollution can cause irreparable damage to our ears when exposed to continuous large sounds like honking of vehicles on a busy road or machines producing large noise in a factory or a mill.
3. **Overpopulation:** Rapid population growth puts strain on natural resources which results in degradation of our environment. Mortality rate has gone down due to better medical facility which has resulted in increased lifespan. More population simple means more demand for food, clothes and shelter. You need more space to grow food and provide homes to millions of people. This results in deforestation which is another factor of environmental degradation.
4. **Landfills:** Landfills pollute the environment and destroy the beauty of the city. Landfills come within the city due the large amount of waste that gets generated by households, industries, factories and hospitals. Landfills pose a great risk to the health of the environment and the people who live there. Landfills produce foul smell when burned and cause huge environmental degradation.
5. **Deforestation:** Deforestation is the cutting down of trees to make way for more homes and industries. Rapid growth in population and urban sprawl are two of the major causes of deforestation. Apart from that, use of forest land for agriculture, animal grazing, harvest for fuel

wood and logging are some of the other causes of deforestation. Deforestation contributes to global warming as decreased forest size puts carbon back into the environment.

6. **Natural Causes:** Things like avalanches, quakes, tidal waves, storms, and wildfires can totally crush nearby animal and plant groups to the point where they can no longer survive in those areas. This can either come to fruition through physical demolition as the result of a specific disaster, or by the long term degradation of assets by the presentation of an obtrusive foreign species to the environment. The latter frequently happens after tidal waves, when reptiles and bugs are washed ashore.

Effects of Environmental Degradation:

1. **Impact on Human Health:** Human health might be at the receiving end as a result of the environmental degradation. Areas exposed to toxic air pollutants can, cause respiratory problems like pneumonia and asthma. Millions of people are known to have died of due to indirect effects of air pollution.
2. **Loss of Biodiversity:** Biodiversity is important for maintaining balance of the ecosystem in the form of combating pollution, restoring nutrients, protecting water sources and stabilizing climate. Deforestation, global warming, overpopulation and pollution are few of the major causes for loss of biodiversity.
3. **Ozone Layer Depletion:** Ozone layer is responsible for protecting earth from harmful ultraviolet rays. The presence of chlorofluorocarbons, hydro chlorofluorocarbons in the atmosphere is causing the ozone layer to deplete. As it will deplete, it will emit harmful radiations back to the earth.
4. **Loss for Tourism Industry:** The deterioration of environment can be a huge setback for tourism industry that rely on tourists for their daily livelihood. Environmental damage in the form of loss of green cover, loss of biodiversity, huge landfills, increased air and water pollution can be a big turn off for most of the tourists.
5. **Economic Impact:** The huge cost that a country may have to borne due to environmental degradation can have big economic impact in terms of restoration of green cover, cleaning up of landfills and protection of endangered species. The economic impact can also be in terms of loss of tourism industry.

Persistent Organic Pollutants (POPs):

POPs are organic chemical substances which are carbon based. They have unique physical and chemical properties that when once released into environment, they travel very long distance through air and water. POPs bio-accumulate in animals and humans.

These pollutants are primarily the products and by-products of human industrial processes.

The initial lists of twelve POPs include;

- Industrial chemicals like polychlorinated biphenyls (PCBs) used in transformer oils;
- pesticides like DDT, endrin, dieldrin, aldrin, chlordane, toxaphene, heptachlor, mirex, hexachlorobenzene (HCB); and
- Unwanted wastes like dioxins ($C_{12}H_4Cl_4O_2$) and furans (C_4H_4O).

They are not soluble in water and are absorbed in fatty tissues where their concentration become magnified by up to 70,000 times the background level. Fish, predatory birds, mammals and humans absorb their greatest concentration and become just like a food chain. When they travel, the POPs travel through them as a result these can be found in people and animals living in regions such as arctic, thousands of kilometer from any major POPs source.

Common Characteristics POPs;

As a general rule, POPs have a number of common properties:

- POPs are persistent in the environment. They resist degradation or breakdown through physical, chemical, or biological processes;
- POPs generally are semi-volatile. They evaporate relatively slowly but when they enter the air, they travel long distances on air currents. They return to earth in rain and snow in the colder areas of the globe, resulting in their accumulation in regions such as the Arctic, thousands of kilometers away from their original sources;
- POPs generally have low water solubility (they do not dissolve readily in water) and high lipid (fat) solubility (they do dissolve easily in fats and oils). Persistent substances with these properties bio-accumulate in fatty tissues of living organisms. In the environment, concentrations of these substances can increase by factors of many thousands or millions as they move up the food chain; and
- POPs have the potential to injure humans and other organisms even at the very low concentrations at which they are now found in the environment, wildlife and humans. Some POPs in extraordinarily small amounts can disrupt normal biological functions, including the

activity of natural hormones and other chemical messengers, triggering a cascade of potentially harmful effects.

Specific Effects of POPs;

Cancer, allergies and hypersensitivity, damage to the central nervous system and peripheral nervous system, reproductive disorders and disruption of the immune system, disruption of endocrine system and hormones which can damage the reproductive and immune systems of exposed individuals as well as their offspring and they can also have developmental and carcinogenic effects.

Green House Effect:

The **greenhouse effect** is the process by which radiation from earth atmosphere warms the earth surface to a temperature above what it would be without its atmosphere. Greenhouse gases in the atmosphere radiate energy, some of which is directed to the surface and lower atmosphere. The mechanism that produces this difference between the actual surface temperature and the effective temperature is due to the atmosphere and is known as the greenhouse effect.

History: The existence of the greenhouse effect was observed first time by **Joseph Fourier** in 1824. The argument and the evidence was further strengthened by **Claude Pouillet** in 1827 and 1838, and reasoned from experimental observations by **John Tyndall** in 1859.The effect was more fully quantified by **Svante Arrhenius** in 1896. However, the term "greenhouse" was not used to refer to this effect by any of these scientists; the term was first used in this way by **Nils Gustaf Ekholm** in 1901. In 1917 **Alexander Graham Bell** wrote "[The unchecked burning of fossil fuels] would have a sort of greenhouse effect", and "The net result is the greenhouse becomes a sort of hot-house. Bell went on to also advocate the use of alternate energy sources, such as solar energy.

Mechanism: Earth receives energy from the Sun in the form of **ultraviolet**, **visible**, and **near-infrared** radiation. Of the total amount of solar energy available at the top of the atmosphere, about 26% is reflected to space by the atmosphere and clouds and 19% is absorbed by the atmosphere and clouds. Most of the remaining energy is absorbed at the surface of Earth. Because the Earth's surface is colder than the photosphere of the Sun, it radiates at wavelengths that are much longer than the wavelengths that were absorbed. Most of this thermal radiation is absorbed by the atmosphere, thereby warming it. In addition to the absorption of solar and thermal radiation, the atmosphere further gains heat by sensible and latent heat fluxes from the

surface. The atmosphere radiates energy both upwards and downwards; the part radiated downwards is absorbed by the surface of Earth. This leads to a higher equilibrium temperature than if the atmosphere were absent.

Within the region where radiative effects are important, the description given by the idealized greenhouse model becomes realistic. Earth's surface, warmed to a temperature around 255 K, radiates long-wavelength, infrared heat in the range of **4–100 μm**. At these wavelengths, greenhouse gases that were largely transparent to incoming solar radiation are more absorbent. Each layer of atmosphere with greenhouses gases absorbs some of the heat being radiated upwards from lower layers. It reradiates in all directions, both upwards and downwards; in equilibrium (by definition) the same amount as it has absorbed. This results in more warmth below. Increasing the concentration of the gases increases the amount of absorption and reradiation, and thereby further warms the layers and ultimately the surface below.

Greenhouse gases, including most diatomic gases with two different atoms (such as carbon monoxide, CO) and all gases with three or more atoms are able to absorb and emit infrared radiation. Though more than 99% of the dry atmosphere is IR transparent (because the main constituents, N_2, O_2, and Ar are not able to directly absorb or emit infrared radiation), intermolecular collisions cause the energy absorbed and emitted by the greenhouse gases to be shared with the other, non-IR-active, gases.

Green House Gases: By their percentage contribution to the greenhouse effect on Earth the four major gases are;

- water vapor, 36–70%
- carbon dioxide, 9–26%
- methane, 4–9%
- ozone, 3–7%

It is not physically realistic to assign a specific percentage to each gas because the absorption and emission bands of the gases overlap (hence the ranges given above). The major non-gas contributor to Earth's greenhouse effect, clouds, also absorb and emit infrared radiation and thus have an effect on the radiative properties of the atmosphere.

Role of Green House Gases in Climate Change: Strengthening of the greenhouse effect through human activities is known as the enhanced (or **anthropogenic**) greenhouse effect. This increase in radiative forcing from human activity is attributable mainly to increased atmospheric

carbon dioxide levels. According to the latest Assessment Report from the **Inter-governmental Panel on Climate Change**, "atmospheric concentrations of carbon dioxide, methane and nitrous oxide are unprecedented in at least the last 800,000 years. Their effects, together with those of other anthropogenic drivers, have been detected throughout the climate system and are extremely likely to have been the dominant cause of the observed warming since the mid-20th century".

CO_2 is produced by fossil fuel burning and other activities such as cement production and **tropical deforestation.** Measurements of CO_2 from the Mauna Loa observatory show that concentrations have increased from about 313 parts per million (ppm) in 1960 to about 389 ppm in 2010. It reached the 400 ppm milestone on May 9, 2013. The current observed amount of CO_2 exceeds the geological record maxima (~300 ppm) from ice core data. The effect of combustion-produced carbon dioxide on the global climate, a special case of the greenhouse effect first described in 1896 by **Svante Arrhenius**, has also been called the **Callendar effect**.

Over the past 800,000 years, ice core data shows that carbon dioxide has varied from values as low as 180 ppm to the pre-industrial level of 270 ppm. **Paleoclimatologists** consider variations in carbon dioxide concentration to be a fundamental factor influencing climate variations over this time scale.

Deforestation: It is the removal of a forest or stand of trees where the land thereafter converted to a non-forest use. Examples of deforestation include conversion of forestland to farms, or urban use. The most concentrated deforestation occurs in tropical rainforests (**Tropical rainforests** are rainforests that occur in areas of tropical rainforest climate in which there is no dry season. All months have an average precipitation of at least 60 mm. About 31 percent of Earth's land surface is covered by forests. The removal of trees without sufficient **reforestation** has resulted in**habitat damage**,**biodiversity loss** and**aridity**. About 31 percent of Earth's land surface is covered by forests.

It is estimated that the world is currently losing over **9 million hectares** per year which is an area the size of **Portugal**.

Causes: According to the United Nations Framework Convention on Climate Change (UNFCCC) secretariat, the direct cause of deforestation is agriculture. Subsistence farming is responsible for 48% of deforestation; commercial agriculture is responsible for 32%; logging is responsible for 14%, and fuel wood removals make up 5%.

Other causes of deforestation may include corruption of government institutions population growth and overpopulation, and urbanization. Globalization is often viewed as another root cause of deforestation.

Environmental Effects:

Atmospheric. Deforestation is a contributor to global warming and is often cited as one of the major causes of the enhanced greenhouse effect. Tropical deforestation is responsible for approximately 20% of world greenhouse gas emissions. Deforestation causes carbon dioxide to emit/spread in the atmosphere. As a result, it produces a layer in the atmosphere that traps radiation from the sun. These radiation converts to heat which causes global warming, which is better known as the greenhouse effect.

Hydrological. The water cycle is also affected by deforestation. Trees extract groundwater through their roots and release it into the atmosphere. When part of a forest is removed, the trees no longer transpire this water, resulting in a much drier climate. Deforestation reduces the content of water in the soil and groundwater as well as atmospheric moisture. The dry soil leads to lower water intake for the trees to extract. Deforestation changes the soil structure which results in flooding and landslides ensue.

Trees and plants in general, affect the water cycle significantly:

- their canopies intercept a proportion of precipitation, which is then evaporated back to the atmosphere (canopy interception)
- their litter, stems and trunks slow down surface runoff
- their roots create macropores, large conduits in the soil that increase infiltration of water
- they contribute to terrestrial evaporation and reduce soil moisture via transpiration
- their litter and other organic residue change soil properties that affect the capacity of soil to store water.
- their leaves control the humidity of the atmosphere by transpiring. 99% of the water absorbed by the roots moves up to the leaves and is transpired.

Soil. Deforestation causes soil erosion (In earth science, **erosion** is the action of surface processes that removes soil, rock, or dissolved material from one location on the Earth's crust, and then transports it to another location). Soils are usually by the presence of trees, which secure the soil by binding their roots to soil bedrock. Due to deforestation, the removal of trees causes sloped lands to be more susceptible to landslides.

Biodiversity. Deforestation on a human scale results in decline in biodiversity and on a natural global scale is known to cause the extinction of many species. Since the tropical rainforests are the most diverse ecosystems on earth and about 80% of the world's known biodiversity could be found in tropical rainforests removal or destruction of significant areas of forest cover has resulted in a degraded environment with reduced biodiversity. It has been estimated that we are losing 137 plants, animals and insect species every single day due to rainforest deforestation, which equates to 50,000 species a year.

Pesticide Residues Estimation from Water

1. Take 500 ml water in 1L separatory funnel.
2. Add 10-15 g NaCl and shake it till the salt dissolve completely.
3. Add 50 ml 15% dichloromethane (85 ml hexane+ 15ml dichloromethane) in hexane
4. Shake the funnel for 1-2 minutes
5. Keep the funnels undisturbed and allow the layers to separate and take out the lower aqueous phase in another 1L separatory funnel.
6. Repeat the process of partitioning two times more using fresh portions of 50 ml of 15% dichloromethane in hexane.
7. Combine the upper organic phase.
8. Pass the extract/combined organic phase through glass funnel containing 2-3 cm layer of anhydrous sodium sulphate for cleaning
9. Concentrate the extract to about 10 ml on rotary evaporator at 45-50 ^{o}C
10. Concentrate the above extract up to near dryness with N_2 streamer
11. Re-dissolve the residues in 5 ml of hexane
12. Evaporate the contents to near dryness for complete removal of dichloromethane
13. Make final volume to 2 ml and submit it for analysis in GC/HPLC/TLC.

Pesticide Residues Estimation from Milk

1. Take 10 ml milk from a homogenized bulk sample
2. Mix it with 10g anhydrous sodium sulphate, 15g silica gel and 5 g florisil
3. Pack the mixture in a glass column in b/w two layers of anhydrous sodium sulphate having a cotton plug at the bottom for cleaning
4. Tap the column gently to ensure compact and uniform packing
5. Add 150 ml of solvent mixture of acetone and dichloromethane with a ratio of 1:1 at a flow rate of 4 ml/mint
6. Divide the extract into two equal parts
7. Add one drop of mineral oil in each part
8. Concentrate the extract to about 10 ml on rotary evaporator at 45-50 ^{o}C
9. Concentrate the above extract up to near dryness with N_2 streamer
10. Re-dissolve the residues in 5 ml of hexane
11. Evaporate the contents to near dryness for complete removal of dichloromethane
12. Make final volume to 2 ml and submit it for analysis in GC/HPLC/TLC.

Pesticide Residues Estimation from Cereals (Jagadish *et al*. 2015)

14. Take 1 kg sample of rice or wheat.
15. Grind the sample to convert it in powder form.
16. Take 25 g of powdered sample in a flask.
17. Add 100 mL mixture of water and acetone (35:65 v/v).
18. keep it for shaking for about 30 minutes in a mechanical shaker and filter it under vacuum through a funnel.
19. Transfer the extract to a flat bottom flask and concentrate it to near dryness in a rotary evaporator.
20. Divide the aqueous extract using 50 mL mixture of hexane: dichloromethane (1:1)
21. Repeat the process twice.
22. Transfer the organic fraction obtained after the partitioning to a 250 mL flat bottomed flask.
23. Filter it through sodium sulphate to absorb the residual moisture.
24. Concentrate the organic portion (supernant) to dryness in a rotary evaporator and re-dissolve it using 10 mL n-hexane: acetone (1:1).
25. Re-dissolve the sample in acetonitrile or ethyl acetate and transfer it into 1.5 ml chemical vials for analysis in HPLC-MS or GC-MS.

Pesticide Residues Estimation from Human Blood/Serum (Araoud *et al*. 2012)

1. Take 2 ml of the serum sample and transfer it into a 15 ml glass vial.
2. Spike the sample with 100 µL of IS solution.
3. Add 1 ml of sodium acetate buffer (3M, pH 4.5) and 9 ml of the solvent mixture (acetone/dichloromethane/hexane) (50/20/30, v/v/v).
4. Mix the solution for 15 min and centrifuge it at 3000 rpm for 5 min.
5. Collect the organic phase (supernant) and evaporate to dryness under a gentle stream of pure nitrogen (N_2).
6. Re-dissolve the residues in 140 µL of ethyl acetate.
7. Place this solution on ultrasound bath for 3 min and centrifuge it at 3000 rpm for 5 min.
8. Finally, inject 1 µL or required amount of this solution into the GC-MS or HPLC-MS system for residues estimation.