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FOOD SAFETY OFFICER

Rajasthan Public Service Commission

Volume - 3

Concerned Subject Part – 2

SIERRA INNOVATIONS

RAJASTHAN FOOD SAFETY OFFICER

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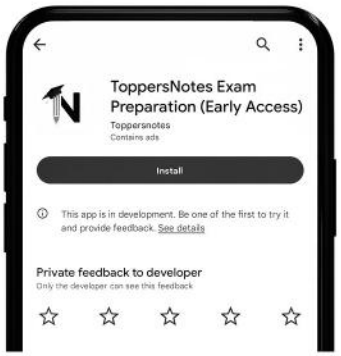

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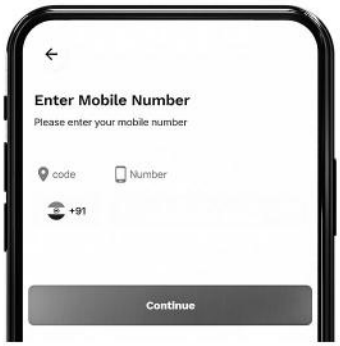

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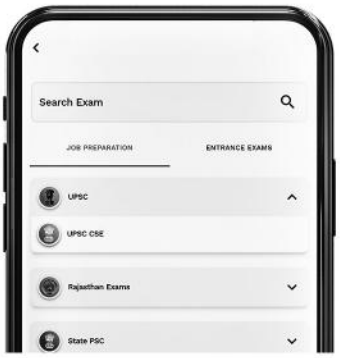

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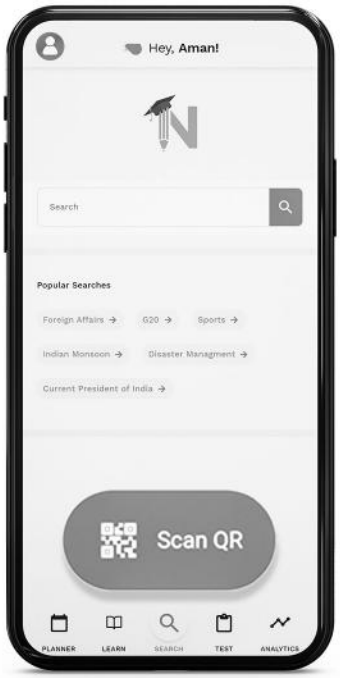

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(GMOs/ Genetically Modified Organisms) -

When any organism is developed by changing its gene (gene alteration), it is called genetically modified organism.

Like Bt cotton, Bt tomato, live cow, rat etc. (Bt Stands for Bacillus thuringiensis and type of bacterial)
You must be aware that whenever a new medicine is discovered, it is given to us for use by scientists only after doing research well after using it on rats and monkeys.
To research the medicines discovered by the scientists whether the medicine works in many or not. To check this, rats are developed in the lab through GMOs.
A few years ago, small tomatoes, cotton, brinjal etc. were used on other types of vegetables, but now big tomatoes, cotton etc. are grown through GMO products.

Transgenic Animals

A genetically modified organism obtained by gene alteration is called a transgenic animal.
Benefits or uses of transgenic animals or GMOs

- Transgenic animals are used in the testing of any drug or vaccine.
- Insect resistant crops are manufactured.
- Vitamin-rich crops are developed.
- Useful food products are made.

In a recent order issued by the Food Safety and Standards Authority of India (FSSAI), the limit of Genetically Modified Organisms (GMO) in food crops imported into India has been fixed at 1%. .

- Earlier in the order issued by FSSAI in August, 2020, on the requirement of Non-GM-Origin-cum-GM-free Certificate by a Competent Authority for 24 food crops imported into the country. Forced
- These are living organisms in which the existing genetic material has been artificially altered in the laboratory using genetic engineering.
- In this, combinations of genes of plants, animals, bacteria and viruses are created, this work is not done through traditional crossbreeding methods.
- In conventional plant breeding, crossing of species of the same genus is done to produce offspring with desired traits of both the parents.
    - A genus is a group of related species. There can be many species in a genus that have similar characteristics or characteristics.
- Bt cotton is the only genetically modified (GM) crop in India. The bacterium Bacillus thuringiensis (Bt) is a foreign gene present in the soil that produces a toxic protein to protect Bt cotton from the common insect pink bollworm.
- Herbicide Tolerant Ht Bt, on the other hand, is achieved by introducing another bacterium found in the soil that enables the strain to resist the common herbicide glyphosate.
- The gene introduced into Bt brinjal enables the plant to resist attack by shoot borers that damage the fruit and shoots.
- Genetic modification in DMH-11 mustard allows cross-pollination instead of self-pollinations.

Transgenic Plants

1. Introduction to Transgenic Plants.
2. Production of Transgenic Plants.
3. Uses

Introduction to Transgenic Plants - The plants in which a functional exotic gene (which is not normally present in that plant) has been inserted by any biotechnological method, are called transgenic plants.

However, many plants carrying genes for economically important traits are either in commercial cultivation or are under sport trials. The term transgenic is used for organisms with foreign genes. These plants which contain foreign genes are called transgenic plants.

Production of Transgenic Plants

Plants in which functional exotic genes (which are not normally present in that plant) have been introduced by any biotechnological method, those plants are called transgenic plants.
However, many plants carrying genes for economically important traits are either in commercial cultivation or are under sport trials. The term transgenic is used for organisms containing foreign genes. Plants that contain foreign genes are called transgenic plants.

There are many methods used in gene transfer.
These include
(i) Electroporation
(ii) Party bombing,
(iii) Micro injection,
(iv) Agrobacterial mediated gene transfer,
(v) Co-cultivation (protoplast transformation) method,
(vi) Leaf disc conversion method,
(vii) Virus Mediated Transformation
(vii) Pollen-mediated conversion,
(ix) Liposome-mediated conversion etc.

The simple method adopted for the production of transgenic plants is as follows -

(i) Locating, identification and isolation of genes important for agricultural economics.
(ii) Selection of a suitable plant transformation vector.
(iii) ligation of the isolated gene into a vector.
(iv) Introduction of transformed vector into plant protoplasts, cells or tissues using various vector transfer techniques.
(v) Culture and differentiation of transformed cells in genetically transformed plants on suitable culture medium.
(vi) Cultures of genetically engineered cells have to be maintained indefinitely to obtain biochemicals.
(vii) Demonstration of integration and expression of foreign genes in transgenic plants with the help of molecular tools.
(vii) Cultivation of transgenic plants.

Uses of Transgenic Plants -

Transgenic plants are produced for the following -

(a) Disease-resistance (against viral, bacterial and fungal pathogens), pest-resistance (against nematodes, insects etc.), tolerance to herbicides and other pesticides
(b) Tolerance to adverse environmental conditions like heavy metal salinity, high or low (cold) temperature b: drought etc.
(c) Variation of floral chromaticity (color of flowers).
(d) To increase the storage period of flowers, fruits and vegetables.
(e) Production of pharmacologically important compounds like insulin, interferons, hormones, blood clotting factors, etc.
(f) Production of vaccines and antibodies in common food items like banana and tomato.
(g) Transfer of Nif genes from bacteria to cereals and other crops.
Transgenic crops with useful traits can be generated. For example, genes coding for insecticidal proteins from Bacillus thuringiensis have been transferred to the cotton plant. This transgenic cotton plant is genetically modified cotton called Bt, which is resistant to black worm.

The transgenic GMO tomato is called Flavr Savr. Its shelf life is longer than that of conventional tomatoes due to delayed ripening process. This is achieved by reducing the amount of the cell wall degrading enzyme polygalacturonase which is responsible for the softening of the fruit.

Legal status of GM crops in India -

The Genetic Engineering Appraisal Committee (GEAC) is the apex body in India that allows commercial production of GM crops.

Using the GM version without approval under the Environment Protection Act, 1986 can lead to a jail term of 5 years and a fine of up to one lakh rupees.

Regulation of imported crops -

Regulation of GMO levels in imported consumer goods was initially done by the Genetic Engineering Appraisal Committee (GEAC).

With the enactment of the Food Safety and Standards Act, 2008, its role was curtailed and FSSAI was asked to grant approval to imported ingredients.

Plant Tissue Culture (PTC)

- It is based on the phenomenon of Totipotency.
- Discovery - By Scientist Haber Land
- Meaning - The ability of cells to divide and differentiate is called Totipotency.
- It is found in animals only in the embryonic stage, that is, after the embryonic stage, cells have the ability to divide only. It does not differentiate, hence this phenomenon is called Totipotency.

Tissue Culture Technique

Laboratory Facility : Dehydration Equipment

(i) Laminar airfllow

(ii) Autoclave Equipment - Dehydrates nutrient medium, equipment and glassware. Condition
upto 15-20 minutes at 120-120°C temperature
15P SI – 15-20 minutes

(iii) Ovan : sterilizes only equipment. That is, dry dehydration is done in it.

Culture Chamber

- The cell of regular atmosphere is called culture chamber.
- Temperature should be $25 \pm 2^{\circ}C$
- Humidity - 60% , Lighting 340 lux
- Nutrient medium: The mixture of different types of nutrients which are necessary for the growth and development of the plant is called nutrient medium.
- Note - Nutrient elements are taken in a certain ratio in the nutrient medium.
- Nutrient medium is prepared by different scientists according to different recipe or protocol.
  - MS Medium,
  - B5 Medium
  - Gamborg Medium,
  - Nitsch Medium
  - White Medium
- The most basic medium - MS Medium – Murashige & Skoog

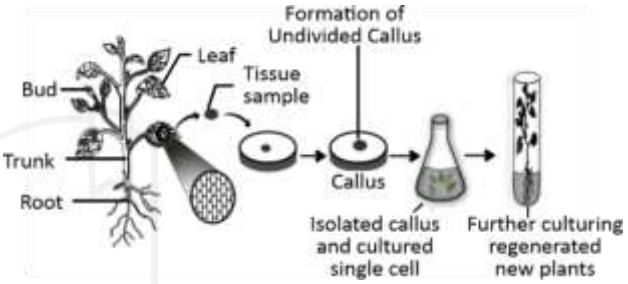
Components of the nutrient medium

Inorganic Components - It is supplied with a variety of properties.

Ex. K^{+},Na^{+},KNO_5,HNO_3 etc.

Organic Component

- They are supplied by vitamins.
  Example: - Nicotinic Acid,
  Thymine ACl
  Ascorbic Acid,
- Carbon source - in the form of sugar
- fructose
- Hormones - Use Auxin, Cytokinin.
- Solidifying agent - to make 1 liter of nutrient medium
- 1 X - use Age
- PH = Medium PA - 5.8
- Dehydrate the nutrient medium by autoclave.
- Temperature – 120°C - for 15-20 minutes and pressure is done at 15 PSI minutes.
- Explant - That part of the plant or tissue which acts as a tissue source in the process of tissue culture are called explants.
  Ex. Root Segment, Stem Segment, Leaf Segment, Local Segment etc.
- Dehydration of Explant: Surface Sterilization happens.
- Alcohol, Naocl – 4-5%,
- After dehydrating the explant, it is kept in dehydrated condition by washing it with dehydrated azat water.

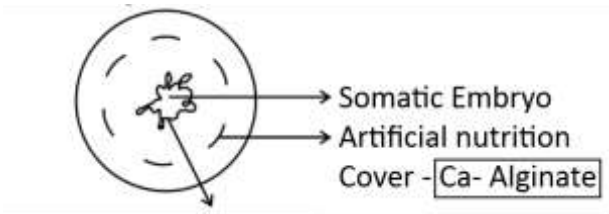


1. Inoculation (Ex- Plantation) - The process of planting dehydrated explants on a nutrient medium is called Inoculation.
   - Protitiration - The process of formation of new tissue by division in plant cell is called Proliferation.
   - Callus - A group of undifferentiated and unorganized cells is called callus.
   - A variety of products are prepared using callus as a tissue source.

   Like: See. Metobolites – Ex- Shikonin
2. Biotransformation – During callus Culture, different types of precursors can be converted into callus by adding different types of precursors to the nutrient medium.
3. Elicitation (Product enhancement) - During callus culture, product enhancing substances are added to the callus medium, which increases the metabolic rate and products.
4. Plant Regeneration - Creation of new plant by plant tissue.
5. By Somatic Embryogenesis - Formation of heart shape or torpedo shape embryo by transferring callus on Embryogenic nutrient medium by tissue Culture method is called Somatic Embryogenesis.
6. New plants can be made by germinating these somatic embryos.

Artificial Seed Production

Synthetic Seed - The structure prepared by enclosing the Somatic embryo in an artificial cover is called synthetic or artificial seed.



- Artificial seeds are produced in seedless plants.
- Protoplasm is preserved with artificial seeds.
- New plants are produced by artificial seed germination.

7. Organogenesis (formation of new plant by organ formation)
   Callogenesis – Shoot formation
   Rhizogenesis – Root formation
8. Generating Hardness and Adaptation: The plantlet prepared by tissue culture method is kept in a controlled environment, it is gradually given adverse conditions due to which adaptation and hardiness are generated. After this the plant can be transferred to the field.

Type of Tissue Culture

- There are two types depending on the nutrient medium.

Static Culture

- The process of culturing on a semi-solid medium containing agar is called static culture.

Suspension Culture

- The process of tissue culture in a liquid nutrient medium, that is, in a medium without agar, is called suspension culture.
- It is necessary to rotate the suspension culture.

Rotate has three purposes.

1. To prevent depression
2. For aeration
3. To break up tissue clumps.

Types of Tissue Culture Based on Ex-plant

(i) Organ/Callus Culture - Any part of the plant can be used as explant and callus can be formed from it.

(ii) Anther/Pollen Culture - In this Anther or pollen is used as ex-plant.

- Through anthor culture, haploid, diploid and multiple plants can be obtained.
- Plants prepared by pollen culture are called androgenic haploid.
- Pollen culture for haploid plants was done by Guha & Maheshwari in India and Shimamura in the world.
- Homozygous diploid plants can be prepared by pollen culture. These can be used in genetic applications.

Gynogenic Haploid

- For this, the haploid cell of female ovary or ovule is used as explant and the plant formed from it is called gynogenic haploid.

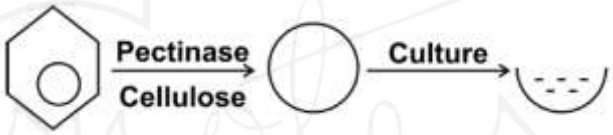
Shoot Tip or Meristem Culture

In this, shoot tip or meristem is used as explant.

- Virus free plants are prepared by this culture.

Embryo Culture

- Using the cell of the overgrown embryo or the cell of the seed as an explant, the development of the embryo is called embryo culture.
- Proto plast Culture
- Prior to Proto plast Culture
- Separation of protoplast is done.
- For protoplast separation, the cell is treated with pectinase and cellulose, which lyses the cell wall and separates the protoplast.



Application of Tissue Culture

- Protoplast Fusin/Somatic Hybridization
- The process of separating protoplasts of two different species, varieties or lineages and fusion them is called protoplast fusion or somatic hybridization.
  Ex. Pomato

Cloning

- Cloning is the process of growth of a cell group, tissue, organ or whole organism by division and differentiation of a single cell. All the cells obtained as a product of this type of process have the same genetic make-up. These cells are exact replicas of each other and are called clones.
- Presently the cloning process is being done keeping in mind three important goals –

(i) Adult DNA Cloning or Whole Organism Cloning

- Through this process, an attempt is made to make the same type of replica from the available organism.

(ii) Embryo Cloning

- Through this process the conversion of monozygotic twins is done.

(iii) Biomedical Cloning

Through this process, fully mature cells are isolated from the embryo of the factor or earlier stage, they are used to culture special types of tissues and organs that can be transplanted back into the person.

- A group of cells derived from the division of a single cell is called a clone. Clone is a Greek word meaning branch, just as all the branches of a tree are the same, similarly all the cells of a clone are the same. Hence clone is a copy obtained by asexual reproduction from one parent (father or mother) which cannot be considered as progeny, the process of making a clone is called cloning. In nature, those organisms such as microorganisms and plants which reproduce asexually form clones.
- The world's most famous sheep 'Dolly' was a clone. Cloning is the method of producing many identical organisms. Dolly was created from a single cell of her mother, she had no father, she had the same genetic characteristics as her mother, as she was a single parent child. In 1997, Dr. Ian Wilmut of Scotland gave birth to the first mammalian cloned sheep named Dolly through cloning.
- The Government of India is insisting on early action to encourage cloning in animals and other animals. The laboratories of the Department of Biotechnology, Government of India, are working in this direction. Other major institutes are - National Institute of Immunology, Center for Cellular and Molecular Biology Hyderabad etc.

Artificial Embryo Twining

- It is a low-tech method of cloning that follows the natural process of reproduction, but unlike the natural process, the process is carried out in a petri dish instead of in the mother's womb. The cells of the developed embryo are isolated at an early stage by the mixing of the sperm and the egg in the Petri dish. These embryonic cells, after growing in a petri dish for a short period of time, are implanted in the womb of the surrogate mother.
- Since twins develop from the splitting of a single fertilized egg, they are both genetically identical.

Somatic Cell Nuclear Transfer-SCNT

- This is the modern technique of preparing clones. Its process is different from artificial embryo twinning, but by this also an organism or clone is prepared. It is also called 'Nuclear Transfer'.
- In this process, the somatic cell is expelled from an organism and its nucleus is removed. After removing the nucleus and all the DNA from the egg cell, the nucleus removed from the somatic cell is inserted into it, so that it starts behaving like a freshly fertilized egg.
- To start the fertilization process, electric waves are passed on them, due to which the cell division starts. Under this process identical clones are obtained by implanting the complete ovum into the womb of the female.

Types of Cloning

Gene Cloning or Molecular Cloning

- Under this, a transgenic microorganism (i.e. transgenic bacteria) is first created by using gene engineering, then clones of that infected bacteria are obtained by creating a suitable environment. They are used in many functions such as human useful protein production (insulin) etc.

Reproductive Cloning

- Under this, an organism is replicated using somatic cell transfer technology or other cloning techniques.

Therapeutic Cloning

- Under this, embryonic stem cells are produced to replace or repair damaged tissues or organs.
- Dolly is the first genetically engineered living organism created by humans.
- First of all, in 1996, Dr. Ian Wilmut and his colleagues cloned a sheep named Dolly using SCNT technology.
- It was the world's first cloned organism developed from a mammal by adult vegetative cells.
- It was developed by conventional cloning techniques and not by 'in vitro fertilization'. Dolly died in 2003 due to lung disease.
- Scientists at National Dairy Research Institute (NDRI) Karnal developed the first buffalo clone 'Samrupa' in 2009 and then 'Garima'. It was here that in 2013 the cloned buffalo 'Garima II' (2010) gave birth to a female calf named 'Mahima'.
- In 2009, the world's first female cloned camel 'Injaz' was developed at the Camel Breeding Center in Dubai.
- The use of cloning has the danger of narrowing the genetic diversity, which can hinder the process of evolution.

Microorganism Cloning

Microorganisms modified by genetic engineering have many uses, such as Keylai, which is useful in the production of human insulin, human growth factor and interferon, while the bacterium, Rhizobium meliloti, is helpful in the incorporation of the 'nif' gene in crops for nitrogen fixation.

Plant Cloning

- There are many orchid clone plants that produce beautiful flowers. Scientists have improved crops through genetic engineering. Such food can also be prepared through gene manipulation, which includes desired properties, such food is called genetically modified food.
- Genetically modified food such as lysine rich pulses and vitamin-A rich rice are becoming the main components of human diet.

Animal Cloning

- Animal cloning is more difficult than plant cloning, but Dr. Ian Wilmut and Roslin Research, Edinburgh, Scotland and his colleagues took mammary cells from a female sheep, placed them in nutrient-free culture media to stop the cells from dividing and to suppress the active gene. .
- The nucleus was isolated from the mammary cell while the oocyte taken from the second host female ewe was delineated. Subsequently, the mammary cell nucleus was fused with the enucleated oocyte by electrical stimulation, thus establishing the nucleus of the mother in this oocyte, which was then implanted into the uterus of the host mother.
- Where she developed into the birth of a lamb and thus Dolly, a genetic replica of the mother, was born. Its first nucleus was taken from the mother (sheep) cell, so genetically it is a replica of the mother sheep. A mother other than the normal or altered egg donor mother in whose uterus the embryo is implanted. That mother is called a surrogate mother.

Environmental Biotechnology

Environmental Biotechnology I.e. Environmental Biotechnology which is used to study the natural environment, it is useful in treating waste water and preventing pollution.

Environmental biotechnology is more efficient at cleaning up waste than conventional methods. Bioremediation is a widespread application employed by environmental engineers to clean up the environment.

These techniques either add nutrients to the soil at the waste site that stimulate bacterial activity or add new bacteria to the soil that digest the waste and clean up the site.

Bioremediation is an interesting area of environmental biotechnology. Its applications are vast and reliable.

Biotechnology applications in food security, agriculture, and climate change and climate mitigation are well known. Industrial insulin hexamer applications of biotechnology are a profitable option for food and agricultural businesses.

In addition, these processes ensure that minimum harm is done to the environment. Today, this branch of Biotechnology is considered to have a major contribution in preventing pollution from the environment.

Bio Pollution and Pollutants

Bio pollution in the present scientific era, the speed with which progress is being made in medicine science, at the same speed new threats are developing for human life. Where in human | On the one hand, creativity is found, while on the other hand destructive tendencies are also increasing in it. Man has become so capable that without using ammunition or arrows-sword, only through bio-pollution, millions of people can be killed. By polluting air, water and food items by bacteria, virus and other micro-organisms like plague fleas etc. causing death to humans is called bio-pollution.

At present this bio-pollution is being used as a bio-weapon. In October 2001, the death of Bob Stevens, photo editor of the United States newspaper Sun, from a disease called anthrax raised bio-pollution to the level of bioterrorism. After this, envelopes contaminated with Annex bacteria started reaching people not only in America but also in India, Pakistan and other countries and the residents of many countries were horrified by this bio-terror. The killing power of germs is also immense. It is more deadly than an atomic bomb. That is why they can also be used as bio-weapons. According to one study, 100 kg. Anyax bacteria can kill 1 million to 3 million people. (If conditions are favorable for bacteria). Whereas a nuclear warhead of one mega ton can kill only 7.5 lakh to 19 lakh people. From 1979 to 1985, 10,000 human lives were lost in Zimbabwe due to bio-pollution.

Due to Biological Pollution

The main cause of bio pollution is the generation of bacteria, viruses and other micro-organisms. These bacteria and viruses are present everywhere in soil, water and air. We all accept the presence of bacteria in the environment. These include bacteria, viruses and fungi. Bacteria are found not only in soil, water and air but also in food items and medicines etc. Some of these bacteria are beneficial and some are harmful. These harmful bacteria are the main factors of bio pollution. For example - polio virus, small pox virus, yellow fever virus etc. Bacterial toxins and viral toxins give rise to different types of infectious diseases like cholera, typhoid, tumor, cancer, lung related diseases and ear and bone infections etc.

Sometimes some such toxic substances are produced in the body of animals or plants which cause terrible effects in humans. In fact, these substances are used by humans by mistake. For example, due to the non-recognition of the person eating the mushroom, poisonous mushrooms can also be used. Toxins are produced in such potatoes in which the kernels are bursting. Many fishes are poisonous which cause poisoning if eaten in place of normal fish. Many common consumer products, such as foods stored in brass or copper utensils, can become toxic if left standing. In this way, substances like disinfectants, bactericides and weedicides etc. also make food poisonous. Many species of bacteria destroy a large amount of food. These bacteria are dead. Therefore, by acting on food items through enzymes, they rot and strangle them. The souring of milk and tolerance of many food items is mostly due to bacteria. Many saprophytic fungi grow on food items and spoil them as well as produce toxic substances which give rise to many types of diseases.

Effects of Bio pollution

Like generated bacteria, viruses and micro-organisms seriously affect the animal and vegetable world. When bio pollution is also being used as a bio weapon. Various deadly diseases can be spread through bio-pollution, such as annex, plague, fleas etc. Annex is basically a disease of animals. It is also called poisonous fever. This disease is spread by a bacterium called Bacillus enthesis. Plague is also a bacterial disease. The name of its bacteria is Yersinia pestis, earlier it used to spread in the form of an epidemic and villages used to end up with this disease, but now it has been controlled. It is spread by fleas (an insect that flies like a mosquito). When the flea bites a mouse infected with the fog, the prong bacteria enter its body. After this, when it bites a human, the human becomes afflicted with the plague. A person suffering from plague dies soon.

Measures to prevent Bio pollution

Bio-pollution is most effective in the modern scientific and technological era. In World War II, Japan spread the disease to some areas of China by spreading contamination with plague-infecting fleas. Therefore, in 1972, resolutions of the Biological Weapons Assembly were signed by various countries to prohibit all types of biological weapons. In 1925, the Geneva Protocol was signed to prohibit chemical and biological weapons. To avoid bio-pollution in India, Defense Research and Development Organization established a laboratory in Gwalior in 1972 AD. The Ministry of Health started the National Disease Surveillance Program in the year 10000. Under this, the vigilance program of the Indian Institute of Communicable Diseases is working. A filter has been prepared by the Defense Research and Development Organization which keeps all the soldiers of the army safe from all kinds of biological and random attacks.

1. Bio magnification

Bio magnification - Some of the toxic substances often present in the waste water of industries can biomagnified in the aquatic food chain. Jab magnification refers to an increase in the concentration of the toxin at successive trophic levels. This is because the toxic substance stored by life cannot be metabolized or excreted and thus moves to the next higher trophic level. That phenomenon is well known for mercury and DDT. Thus, the concentration of DDT increases at successive trophic levels. If this concentration in water starts from 0.003 ppv (ppb = parts per Billion) then it eventually increases to 25 ppm in fish and birds through bio magnification. In birds, high concentration of DDT impairs calcium metabolism, causing thinning of the egg shell and premature cracking, leading to a reduction in the bird population.

2. Bioremediation

Bioremediation is a process used to remediate contaminated media including water, soil and subsurface materials by altering environmental conditions to inhibit the growth of microorganisms and degrade target pollutants. Cases where bioremediation is commonly seen are oil spills, soil contaminated by acidic mining drainage, underground pipe leaks, and crime scene cleanup. These toxic compounds are metabolized by enzymes present in microorganisms. Most bioremediation processes involve oxidation-reduction reactions, where either an electron acceptor (usually oxygen) is added to encourage oxidation of a reduced contaminant (such as a hydrocarbon) or an electron donor (usually an organic substrate) is added to reduce oxidized pollutants (nitrate perchlorate, oxidized metals, chlorinated solvents, explosives, and propellants). Bioremediation is used to reduce the effects of byproducts created from human-generated activities such as industrial and agricultural processes. In many cases, bioremediation is less expensive and more sustainable than other treatment options. Other remediation techniques include thermal absorption, desorption, verification, air stripping, bioleaching, rhizofiltration and soil washing. Biological remediation is a similar approach used to treat wastewater, including wastewater, industrial wastewater, and solid waste. The ultimate goal of bioremediation is to remove or reduce harmful compounds to improve soil and water quality.

Contaminants can be removed or reduced with different bioremediation techniques which is what Saudi X-Seed is all about. Bioremediation techniques are classified based on the location of treatment. These eco-techniques treat contaminated sites in a non-destructive manner and are cost-effective. Whereas, X-ray techniques usually require excavation of the contaminated site which increases the costs. In both of these approaches, additional nutrients, vitamins, minerals, and pH may be added to create optimal conditions for the microorganisms. In some cases, special microbial cultures are added to further enhance biodegradation (bio stimulation). Some examples of bioremediation related technologies are Phytoremediation, Bioventing, Bio attenuation, Biosparging Contaminants, Composting (Bio piles and Windrows), and Land farming.

Biomagnification

- Biomagnification, also known as Blomagnification or biological magnification, is any concentration of a toxin, such as pesticides, heavy metals, in the tissues of tolerant organisms at successively higher trophic levels in a food chain.
- This increase may result from the presence of persistent compounds in the environment that cannot be broken down by environmental processes.
- These chemicals have the potential to move progressively up the food chain.
- They are not expelled or excreted by the organisms that encounter them. Furthermore, they are insoluble in water so tend to be stored in adipose tissue.

Process

- Pesticides or heavy metals → enters lakes, rivers and oceans → phytoplankton/zooplankton → eaten by fish → which in turn are eaten by larger fish → fish are eaten by larger birds → animals → humans.
- As they move up the chain, these substances become increasingly concentrated in tissues/fat or internal organs.
- If the organism lacks enzymes to break them down. When eaten by another organism, the fat is absorbed in the gut, carrying the substance, which is then stored in the predator's fat.
- Since there is a lot of energy loss at each level of the food chain, a predator must consume many preys including all their lipophilic substances.
- Two common groups known to biomagnify are chlorinated hydrocarbons, also known as organochlorines, and inorganic compounds such as methylmercury or heavy metals.
- Both are lipophilic and do not degrade easily.

Example

Mercury

- Mercury is only present in small amounts in seawater, is absorbed by algae (usually in the form of methyl mercury) and can reach higher trophic levels.
- Thus, predatory fish such as swordfish and sharks or birds such as osprey and eagle have high concentrations of mercury in their tissues.
- For example, herring contains about 0.01 parts per million (ppm) of mercury and shark contains more than 1 ppm.

DDT

- When an animal consumes food containing DDT residues, DDT progressively accumulates in the animal's adipose tissue through a process called bioaccumulation.
- The higher an animal is on the food chain (for example a tertiary consumer such as a seal), the higher it is called biomagnification.
- The process resulted in high levels of DDT in their bodies.
- In birds i.e. eagles and falcons, the egg shell becomes thinner due to high concentration of DDT.
- PCBs (Polychlorinated Biphenyls)
- PCBs are highly carcinogenic chemical compounds, formerly used in industrial and consumer products.
- Ganga, the national river, was found to be polluted with five heavy metals – chromium, copper, nickel, lead and iron.
- Plastic contains a harmful chemical called Bisphenol A which is one of the major pollutants released into water bodies.
- Other examples are -
- Hexachlorobenzene (HCB), Toxaphene, Monomethylmercury, all can be biomagnifying and lyophilic in nature.
- Other persistent organic pollutants may also increase.
- effects of biomagnification
- effects on human health
- Mercury, cadmium, lead, cobalt, chromium, and other chemical poisons make people more vulnerable to cancer, liver and kidney failure, respiratory illnesses, birth defects in pregnant women, brain damage, and heart disease.
- Mercury and polycyclic aromatic hydrocarbons-contaminated seafood, for example oysters, have been linked to diseases such as hepatitis and cancer (PAH).
- Effects on reproduction and development of marine organisms.
- Accumulation of hazardous substances and elements in vital organs of aquatic species affecting their reproduction and development.
- Seabird eggs, for example, have thinner shells than usual, which birds may break instead of hatching their eggs.
- Selenium and other heavy metals, such as mercury, have a negative effect on fish reproduction by destroying their reproductive organs.
- In addition, PCBs (polychlorinated biphenyls) inhibit biomagnification and reproduction, and are found in high concentrations in aquatic systems.
- Destruction of coral reefs.
- Cyanide, which is used in gold leaching and fishing, destroys coral reefs.
- Various marine creatures use the reef as a base for spawning, feeding and living.
- When aquatic species are destroyed, their existence is in danger.

Disruption of Food Chain

- The survival of many aquatic species is dependent on the natural food chain.
- When chemicals and other pollutants are transferred to soil, rivers, lakes or oceans and taken up by diverse creatures, the linked links within the food chain are disrupted.
- This occurs when small animals consume toxic elements or absorb plants, which are then eaten by larger organisms, causing damage to the entire natural food chain.
- Humans and top animals in the food chain can consume organisms or plants contaminated with compounds such as mercury, copper, chromium, selenium and cobalt, putting them at risk of disease, reproductive problems and even death.

Persistent Organic Pollutants

- Sometimes referred to as "forever chemicals", are organic compounds that are resistant to environmental degradation through chemical, biological, and photolytic processes.
- They are toxic chemicals that adversely affect human health and the environment around the world. Because they can be carried by air and water, most PoPs originating in one country can affect people and wildlife far away from where they are used and released.
- The "dirty dozen" PoPs identified by the Stockholm Convention include aldrin, chlordane, dieldrin, endrin, heptachlor, HCB, mirex, toxaphene, PCBs, DDT, dioxins, and polychlorinated dibenzofurans.
- They bioaccumulate in the fatty tissues of living organisms.
- The compounds that make up PoPs are also classified as PBt (persistent, bioaccumulative and toxic) or TOMP (toxic organic micropollutants).

Description	Biomagnification	Bioaccumulation
Definition	Chemical compounds or toxins accumulate at higher trophic levels of the food chain. In other words, animals have higher concentrations of chemical compounds.	Toxic substances are formed in the tissue of a certain organism.
Food chain	Biomagnification occurs between two trophic levels.	Bioaccumulation, like biomagnification, occurs at the trophic level.
Cause	As one moves up the food chain, the number of steps increases.	An increase in the concentration of a substance within an organism.
Pollutant Concentration Level	Causes the concentration of pollutants to increase in an organism.	As they move from one trophic level to another trophic level, the toxicant concentration increases.
Example	Pollutants and other toxins absorbed by microscopic aquatic animals are carried to smaller fish, which are then consumed by larger fish and other aquatic species	Mercury is formed in aquatic animals.

Bioremediation

- Bioremediation is a biotechnical process, which abates or cleans up contamination.
- It is a type of **waste management** technique which involves the **use of organisms** to **remove** or utilize **the pollutants** from a polluted area.
- It is a "**treatment that uses naturally occurring organisms to break down hazardous substances into less toxic or non-toxic substances**".
- There are several remedies where contaminated water or solid is purified by chemical treatment, incineration, and burial in a landfill.
- There are other types of waste management technique which include solid waste management, nuclear waste management, etc. **Bioremediation is different as it uses no toxic chemicals**.

Phytoremediation

- Plants are directly used to clean up or contain contaminants in the soil.
- This method of bioremediation will help mitigate the environmental problem without the need to excavate the contaminant material and dispose of it elsewhere.

Microbial Bioremediation

- Microorganisms like Bacteria and Fungi are the main role player when it comes to executing the process of Bioremediation.
- Bacteria are the most crucial microbes in this process as they break down the waste into nutrients and organic matter.
- Even though this is an efficient process of waste management but bioremediation cannot destroy 100% contaminants.
- Bacteria can easily digest contaminants like chlorinated pesticides or clean oil spills but microorganisms fail to destroy heavy metals like lead and cadmium.
- These techniques have been successfully used to remediate soils/sludges & groundwater contaminated by petroleum hydrocarbons, solvents, pesticides, wood preservatives, and other organic chemicals.

This Technology Includes

- **Biostimulation** (stimulating viable native microbial population),
- **Bioaugmentation** (artificial introduction of viable population of microbes),
- **Bioaccumulation** (live cells),
- **Biosorption** (Biosorption is the removal of **heavy metals**, compounds and particulates from a solution **by low cost** biological materials such as **dead mass or natural materials** with greater degradative ability),
- **Phytoremediation** (plants)
- **Rhizoremediation** (plant and microbe interaction and removal of contaminants through roots.)
- **Bioventing**
- **Bioleaching**
- **Land farming**

Types of Bioremediation

1. **Biostimulation**
   - As the name suggests, **the bacteria is stimulated** to initiate the process.
   - The **contaminated soil** is first **mixed with special nutrients** substances including other vital components either in the form of **liquid or gas**.
   - It **stimulates the growth of microbes** thus resulting in **efficient and quick removal of contaminants** by microbes and other bacteria.
2. **Intrinsic Bioremediation**
   - The process of intrinsic bioremediation is **most effective in the soil and water** because of these two biomes which always have a high probability of being full of contaminants and toxins.
   - The process of intrinsic bioremediation is mostly **used in underground places like underground petroleum tanks**.
   - In such place, it is difficult to detect a leakage and contaminants and toxins can find their way to enter through these leaks and contaminate the petrol.
   - Thus, only microorganisms can remove the toxins and clean the tanks.
3. **In situ bioremediation**
   - **In situ** — it involves treatment of the contaminated material **at the site**. It includes:
   - **Bioventing:**
     - **Supply of air and nutrients through wells** to contaminated soil to stimulate the growth of indigenous bacteria.
     - It is used **for simple hydrocarbons** and can be used where the contamination is **deep under the surface**.
   - **Biosparging**:
     - **Injection of air under pressure** below the water table **to increase groundwater oxygen concentrations**
     - And enhance the **rate of biological degradation of contaminants by naturally occurring bacteria**.
   - **Bio augmentation**:
     - **Microorganisms are imported** to a contaminated site to enhance degradation process at sites where soil and groundwater are contaminated with **chlorinated ethenes**, such as **tetrachloroethylene** and **trichloroethylene**.
     - Bioaugmentation is used to ensure that the in-situ microorganisms can completely **degrade these contaminants to ethylene and chloride, which are non-toxic.**
   - **Bioleaching**:
     - It is **the extraction of metals from their ores through the use of living organisms**.
     - This is **much cleaner** than the traditional heap leaching using cyanide.

Advantages of in situ bioremediation

- No need to excavate & transport soils - typically **less expensive**
- Can treat a large volume of soil at once.
- Causes **less contaminants** to be released than ex-situ techniques
- Creates **less dust**
- Most effective if permeable sandy soil (uncompacted)

Disadvantages of in situ Bioremediation

- **Least effective in clays/highly layered subsurface environment**s - oxygen cannot be evenly distributed throughout the treatment area.
- May be slower to reach clean-up goal (if less easily degradable contaminant, requires years).
- May be more difficult to manage (than ex situ techniques).

4) Ex-situ bioremediation (outside/away from site**)**

- Ex situ techniques include: slurry & solid phase bioremediation:
- **Solid-phase soil treatment processes** include **land farming**, **soil bio piles**, and **composting**.
- **Slurry-phase soil treatment processes** include the **slurry phase bio-reactor.**
- **Bioreactors**:
  - These are **large vessels** where the contaminated material can be monitored and conditions for bioremediation can be controlled.
  - Biological organisms typically have conditions where they operate best.
  - In bioreactors we **can control the mixing rate**, temperature, pH, and nutrient levels to suit the organisms breaking down our contaminant.
- **Land farming**:
  - It involves spreading contaminated soil into a lined bed (to prevent leaching) and periodically applying nutrients and mixing the soil to boost biological activity.
- **Bio piling**:
  - It places the contaminated **soil into pile**s that are well aerated and nutrients are added to speed up bioremediation.
  - In all cases, the contaminant levels are monitored to verify that bioremediation is taking place and steps are taken to ensure that contaminated material stays out of contact with the environment.

Advantages & Disadvantages of ex situ Bioremediation

- **Ex situ techniques can be faster**, easier to control, and used to treat a wider range of contaminants and soil types than in situ techniques.
- There is more certainty about the **uniformity** of treatment because of the ability to homogenize, screen, and continuously mix the soil.
- However, they require excavation of soils, leading to **increased costs** and engineering for equipment,
- More **risk of material handling**/worker exposure conditions.
- Usually **requires treatment of the contaminated soil** before and, sometimes, after the actual bioremediation step.

Applications with Examples (important for exam)

- Bio-Informatics Division at Indian Institute of Chemical Biology (CSIR-IICB), Kolkata, isolated a fungus, **Arthriniummalaysianum,** and used the fungus biomass to remove **hexavalent chromium Cr(VI) metal**.

Uses of Microorganisms for waste Treatment

- **In the presence of oxygen**.
  - **Examples** of aerobic bacteria recognized for their degradative abilities are **Pseudomonas, Alcaligenes, Sphingomonas, Rhodococcus, and Mycobacterium**.
  - These microbes have often been reported to **degrade pesticides and hydrocarbons**, both alkanes and compounds.
  - Many of these bacteria use the contaminant as the sole source of carbon and energy.
- **In the absence of oxygen**.
  - Anaerobic bacteria are not as frequently used as aerobic bacteria.
  - There is an increasing interest in anaerobic bacteria used for bioremediation of polychlorinated biphenyls (PCBs) in river sediments, dechlorination of the solvent trichloroethylene (TCE), and chloroform.
- **Ligninolytic fungi**.
  - Fungi such as the **white rot fungus Phanaerochaete chrysosporium** have the ability to degrade an extremely diverse range of persistent or toxic environmental pollutants.
  - Common substrates used include straw, saw dust, or corn cobs.
- Aerobic bacteria that grow utilizing methane for carbon and energy.
  - The **initial enzyme** in the pathway for aerobic degradation, **methane monooxygenase**, has a broad substrate range and is active against a wide range of compounds, including the **chlorinated aliphatics**.
- **Bioremediation can be used**
  - To **degrade highly toxic heavy metals**, chemicals, effluents.
  - To **remove Heavy metals from tanneries**, and to treat **Oil spills**.
  - Hence, for the processes harmful for the humans, microorganisms may be used to reduce the toxic compounds to less toxic ones.

- Numerous members of **Pseudomonas** have also been modified with the **lux gene**, for the detection of **the polyaromatic hydrocarbon naphthalene**.
- **China-coal waste water plant**: contains thiocyanate, phenols, ammonia and sulphur, treated with **Peudomonas stutzeri**.
- **Diesel** contaminated soil can be treated with inoculation of Mycelium of **Oyster Mushroom** (Pleurotus ostreatus).

My Coremediation

- Bioremediation using Fungi.
- **Mycelium contains extracellular enzymes** and acids for remediation. **Fungi can degrade wood**.
- e.g., **white rot & brown rot fungi**. **Oyester mushroom Pleurotus ostreatus** can turn **95% of poly hydrocarbons into non-toxic form.**
- **Sulphate-reducing bacteria** such as Desulfovibrio desulfuricans have the ability to convert sulphate to hydrogen sulphate which then reacts with heavy metals such as **Cd and Zn** to form insoluble forms of these metal sulphides.
- **Yeast (Sacharomyces cerevisiae)** are also used as efficient agents of bioremediation because they have the ability to remediate toxic metals from contaminated wastewaters **by biosorption** through the **mechanism of ion exchange.**
- **Methanogenic, Methanococcus, methanobacterium, microbes are used in Sludge digestion stage of wastewater treatment**
- **Slurry-phase bioremediation technique** includes mixing contaminated water and soil, fertilizers and carbon dioxide in a bioreactor to stimulate biodegradation
- **Ananda Chakraborty received the first U.S. patent for a GM entity** for Pseudomonas engineered to degrade petroleum
- Deinococcus radiodurans **bacterium can withstand the dosage of radiation, which are several times higher than what human cells can tolerate**

Collection of Data

Types of Data

1. Primary data
2. Secondary data

Methods of collecting primary data

1. **Direct Personal Research**
   In this method, the researcher himself establishes direct contact with the informants. This method is suitable for the following researches.
   - Whose area is limited and local in nature.
   - Where the data has to be kept secret.
   - There should be more emphasis on the originality of the data.
   - Where personal presence is required.
2. **Indirect Oral Research**
   Under this method, information is not obtained from persons having direct relation, rather information is obtained from third party persons who are indirectly aware of the situation.
   For example, instead of asking the laborers themselves about the living condition of the laborers, asking the labor unions or the owners of the mills.
   This method is suitable in the following circumstances –
   - The scope of research should be wide.
   - There should be no personal contact with direct informers.
   - The informant is not interested in giving the information.
   - The data should be of complex nature.
3. **Getting information from local sources by correspondents**
   In this method, researchers appoint local correspondents at different places of research and they are instructed to send necessary information regularly, on the basis of which they give information to the researchers from time to time.
   This method is suitable in the following situations -
   - Information has to be received regularly for sufficient time.
   - There should not be a need for a high level of "purity".
   - Estimates and trends have to be known.
4. **Information receipt / post by filling the schedules by the informers**
   Under this method, the researcher prepares a schedule of related questions keeping in mind the purpose of the research, then prepares copies of that schedule and sends them by post to the informers, who fill it and return it to the researcher in the stipulated time. This method is used in the following areas -
   - Be the area of detailed research.
   - Where the people are literate.
   - Research of consumers interests, market survey is done under this.
   - This method is used for the annual survey of industries.
5. **Receipt of information by filling schedules by enumerators**
   Under this method, schedules are prepared keeping in mind the various aspects of the research, and after dividing the area of research into several parts, enumerators are appointed for each part, who go door-to-door and inquire about the indicators and prepare the schedules themselves.

Collection of Secondary Data

1. **Published Sources**
   (i) International publication.
   (ii) Government publication.
   (iii) Publications and reports of semi-government organizations.
   (iv) Reports of committees and commissions.
   (v) Reports of business organizations.
   (vi) Research work of universities.
   (vii) Newspapers.
   (viii) Market news.
   (ix) Individual Researcher.
   (x) Publications of associations and organizations.
2. **Unpublished Source**
   Secondary data also become available in unpublished form. Researchers collect material for various purposes which are not published. Unpublished materials are for the personal use of individuals or members of trade associations only.

Census and Sample Investigation

Census Investigation

If information is collected about each unit of the whole, then such research is called computational research.

Sample Research

If information is collected about only some representative units out of all the units of the whole, then it is called sample research.

Method of Sampling

1. **Deliberate Sampling**
   In this method, the researcher, on the basis of his knowledge, training, skill and experience, selects a sample of some such units which, in his opinion, represent the characteristics of the whole.

2. Random Sampling

In this method, the units of the population are properly sorted such that each unit has an equal chance of being included in the sample. The units in the sample are selected completely on the basis of probability or chance.

Random Sampling Methods

- Lottery Method
- Drum Method (By Rotating Drum)
- By random numbers
- By Systematic Random Sampling

1. Mixed Sampling

In this category, such methods are included which are based on a combination of deliberate sampling method and random sampling method.

In this method the following are included –

- Stratified Sampling – both preconceived and random are included.
- Multistage sampling - suitable for sampling a very large area.
- Multistage Sampling - Samples are taken by random sampling method.
- Group Sampling - In this the population is divided by size, qualities, areas.

Probability Theory and Random Sampling

Random sampling is based on probability theory. Probability is based on the probability of occurrence or non-occurrence of an uncertain event.

$$\text{Probability of occurrence of event} = P = \frac{M}{M+N}$$

$$\text{Probability of event not happening} = q = \frac{M}{M+N}$$

Probability = Number of favorable events / Total number of all events

Note – The value of probability is (1) in certain situation and (0) in uncertain situation.

Law of Mass Inertia

This rule is also known as the principle of stability of large data. This rule states that there is more stability in large groups than in small groups.

Editing of Collected Data & Statistical Error

Types of statistical fallacies

1. Biased error

2. Unbiased error

1. Biased Error

The error that are caused by the bias of the informers or due to the fault of the measuring instruments are called biased illusions.

These errors keep increasing in the same direction. Hence, they are also called cumulative illusions.

For example, young women deliberately tell less about their age.

Causes of biased error

(i) Informant bias

(ii) Enumerator's fault

(iii) Orientation of sample

(iv) Faulty parameter

(v) Misinterpretation

2. Unbiased Error

These error arises without any bias. These illusions arise accidentally in the data due to carelessness. These are also called compensatory error.

Measure of statistical fallacy

(i) Absolute Error

(ii) Relative Error

(i) Absolute Error

- The difference between the actual value and the estimated value is called absolute error.
- If the actual value is greater than the expected value, it is not a positive but a negative illusion.

A.E. = a-e

a=Actual Value

e= Estimated Value

(ii) Relative Error

Relative error (R.E.)

$$= \frac{\text{Actual Error}}{\text{Estimated Value}} \text{ or } \frac{\text{A.E.}}{e} = \frac{a-e}{e}$$

Percent Error - If the relative error is multiplied by 100, the result obtained is called the percentage error.

$$\frac{\text{A.E.}}{e} \times 100$$

Actual Value = ?

Example = relative error = 0.75, absolute error 60

$$\text{Relative error} = \frac{\text{A.E.}}{e} = 0.75 = \frac{60}{e} = e = \frac{60}{75} \times 100 = 80$$

Actual Value $80 + 60 = 140$

Estimation Errors

- When the error bias is absolute = Avg. Absolute error X N

$$\text{Relative} = \frac{\text{Avg. Absolute error} \times N}{\text{Eastimated Value}}$$

When illusions are neutral