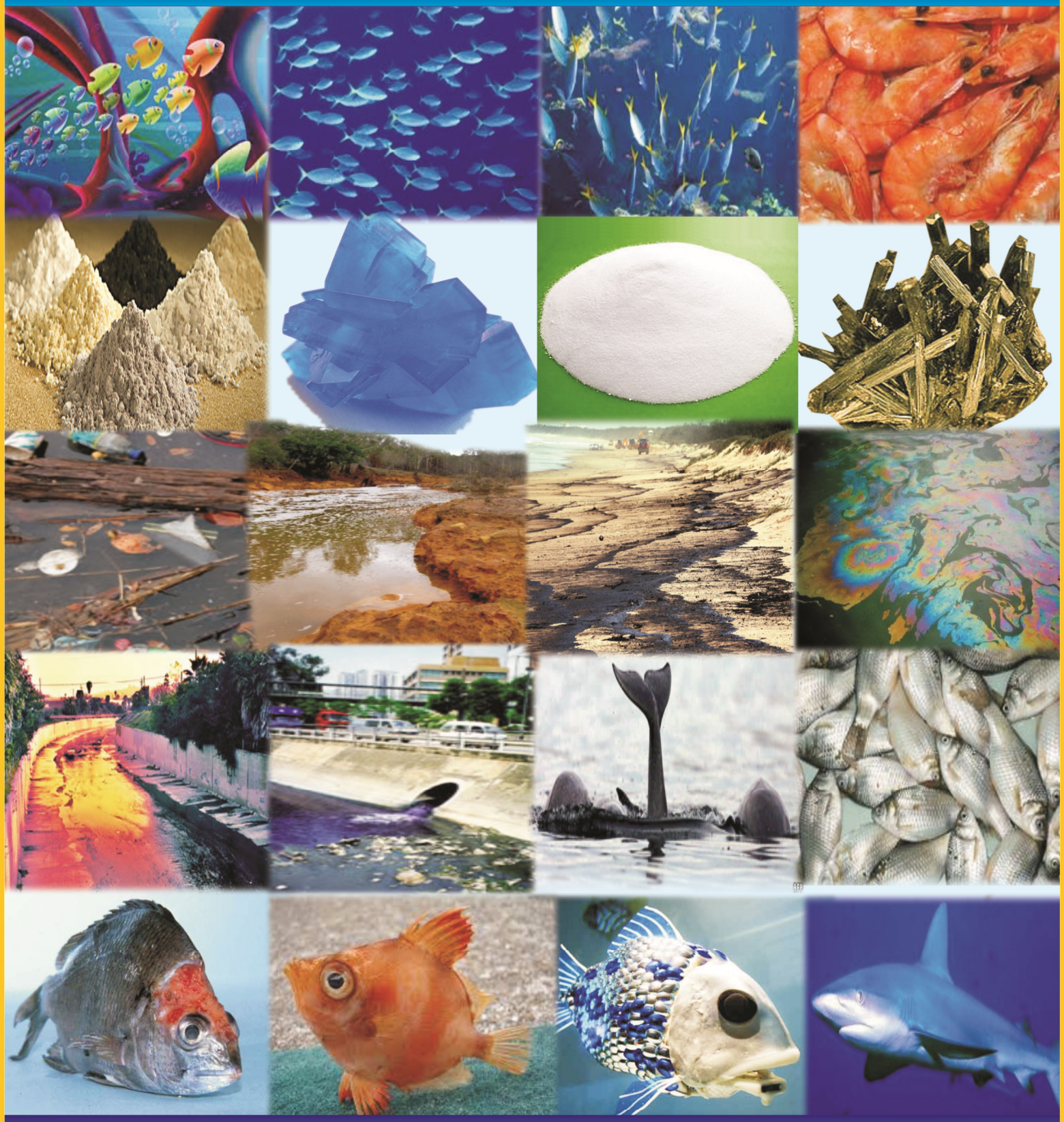
HEAVY METALS POISONING IN FISH

Dr. Govind Pandey



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HEAVY METALS POISONING IN FISH

(Manual on Aquatic Toxicology)

By

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PREFACE

"Aquatic Science" is the multidisciplinary study of *'aquatic ecosystem'* that deals with both freshwater and marine water systems. *"Aquatic environment"* is the interacting system of resources, e.g., water and biota. *"Aquatic animals"* are either vertebrate or invertebrate that live in water for most or all of their lives. *'Vertebrate aquatic animals'* are *'fish'* that are mostly vertebrates or animals having backbones. A *"fish"* is any gill-bearing aquatic vertebrate (or craniate) animal that lacks limbs with digits. A *'pollutant/contaminants/toxicant/toxic substances/hazardous substance'* is one which can cause harm to the environment or life.

Industrialization of the modern world has led to the proliferation of many pollutants or toxic metals in our environments. The toxic substances are emitted to water bodies both directly and indirectly through diffuse and point sources from a wide range of land-based and marine sources, including agriculture and aquaculture, industry, oil exploration, mining activities, transport, shipping and waste disposal, as well as our own homes. The range of potential toxic substances is very vast and includes inorganic poisons, organic poisons, heavy metals, polychlorinated biphenyls and pesticides. *"Heavy metal"* is any metallic chemical element which has a relatively high density, and is *'toxic or poisonous'* to humans, animals or aquatic ecosystem at low concentrations. Excessive concentrations of heavy metals in biological systems are detrimental to body. They destabilize ecosystems because of their bioaccumulation in organisms, and produce toxic effects on the biota and even death of living organisms.

Considering the above grounds in view, this manual, **"Heavy Metals Poisoning in Fish"** has been put forth to explore out the important *'heavy metals'* with regard to their environmental pollution and toxic effects on the *'fish and other aquatic environment/ ecosystems'*. The important *'toxic heavy metals'* described in this manual include mercury, arsenic, lead, cadmium, copper, iron, molybdenum, zinc, manganese, antimony, chromium and nickel. Indeed, this manual is a part of *'Aquatic Toxicology'*, especially devoted to *'Fishery and Veterinary Sciences'* from which the fishery/veterinary students, teachers and scientists can achieve the useful knowledge of heavy metals toxicity occurring in fish and other aquatic organisms.

This apart, I pay my sincere thanks to Dr. A.B. Shrivastav (Director, Centre for Wildlife Forensic & Health); Dr. Y.P. Sahni (Director, Research Services); and Dr. Madhuri Sharma (Associate Professor, College of Fishery Science), Nanaji Deshmukh Veterinary Science University, Jabalpur (MP, India). I also gratefully acknowledge to all the authors/publishers/books/websites from where the matters and figures have been taken and incorporated in this fruitful manual on **"Heavy Metals Poisoning in Fish"**.

26th November, 2013 **Dr. Govind Pandey**

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AQUATIC SYSTEM AND FISH

AQUATIC SCIENCE AND AQUATIC SYSTEM

'Aquatic Science' is the multidisciplinary study of *'aquatic system' ('aquatic ecosystem')*, encompassing both freshwater and marine water systems. The investigations usually examine the human impact on and interaction with aquatic systems and range in scale from molecular level of contaminants to stresses on entire ecosystems. Main areas of study under aquatic science are hydrology; limnology (study of lakes, rivers, wetlands and groundwater); biogeochemistry; aquatic ecology; marine biology; and oceanography. *'Ecology'* is the study of relation of living organisms with each other and their surroundings. *'Ecosystem'* is community or network of individuals, feedback between the living (biotic) and non-living (abiotic) components of an environment. Thus, an *'aquatic ecosystem'* is an ecosystem in a body of water. On the other hand, *'aquatic ecosystem'* refers to the water bodies and biotic communities. The communities of organisms that are dependent on each other and on their environment live in the aquatic ecosystems. A *'pond ecosystem'* is a self-regulating and self-sufficient freshwater ecosystem. *'Freshwater'* is stagnant, receiving water during rainy season (Wikipedia, 2013a).

Types of Aquatic Ecosystem:

Two main types of aquatic ecosystems (Fig. 1-2) are as under (Wikipedia, 2013a):

1. ***Freshwater Ecosystem-*** This is the freshwater environment (with no salinity or salt concentration). It covers 0.8% of the earth's surface and inhabits 0.009% of its total water. The freshwater ecosystem contains 41% of the world's known fish species. It has two categories-

   (a) Lentic ecosystem- This is slow-moving water, including pools, ponds and lakes.

   (b) Lotic ecosystem- This is rapidly-moving water, e.g., streams and rivers.

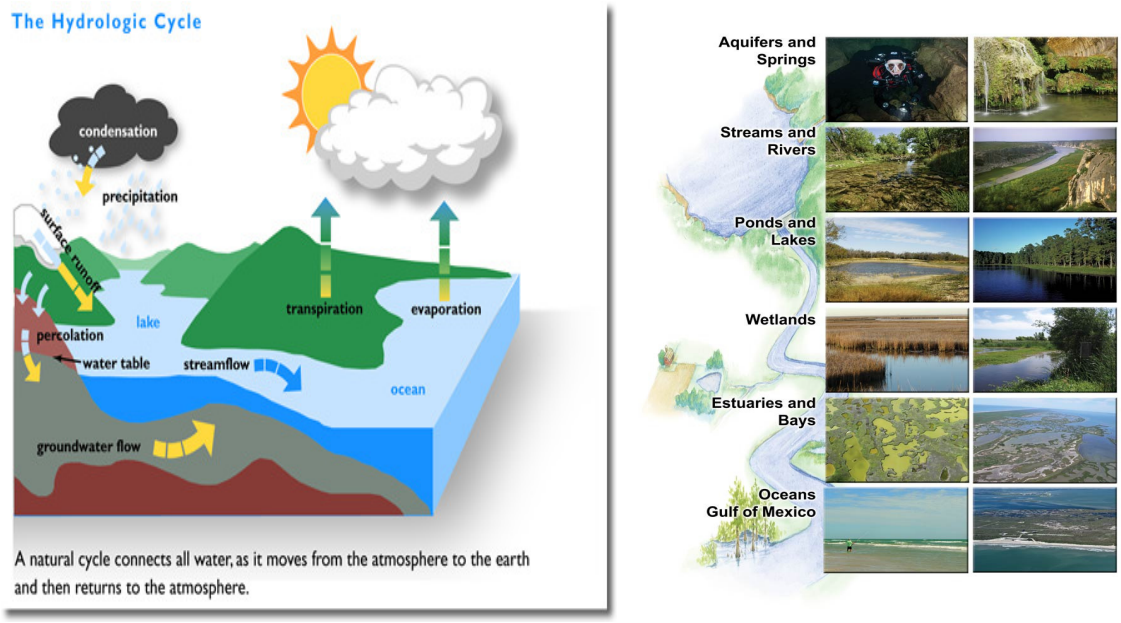

Fig. 1-2: Aquatic Ecosystems

[Source of figures: Different websites which are gratefully acknowledged]

2. ***Marine Ecosystem-*** This ecosystem is characterized by the saline water environment, which covers about 71% of the earth's surface. The marine ecosystem is distinguished from freshwater ecosystem by the presence of dissolved compounds, especially salts in the water. About 85% of dissolved materials in seawater are sodium (Na) and chloride (Cl). Seawater has an average salinity of 35 parts per thousand (ppt) of water. Actual salinity differs among various marine ecosystems. The zones of marine ecosystems may be-

   a) *Oceanic zone*- It is the open part of ocean where whale, shark and tuna, etc. live.

   b) *Profounder zone*- It is the part at the bottom or deep water.

   c) *Benthic zone*- It is the bottom substrate.

   d) *Intertidal zone*- It is the area between high and low tides.

   e) *Coral reef*- It is the underwater structure made from calcium carbonate ($CaCO_3$) secreted by corals (colonies of tiny living animals found in marine environment).

   f) *Hydrothermal vent*- It is the environment where chemosynthetic bacteria form food base.

   g) *Organisms of marine environment*- It includes the organisms like algae, dinoflagellates, corals, finfishes, cephalopods, echinoderms, etc.

h) *Estuary*- It forms a transition zone between river and ocean environments.

i) *Salt marsh*- It is the upper coastal intertidal zone between land and brackish water (saltwater).

Working of Ecosystem and Food Web:

Energy from sun is the driving force of an ecosystem. This light energy is captured by *'primary producers'* (mainly green plants and algae) and converted by a process called '*photosynthesis'* into chemical energy (e.g., carbohydrates). The chemical energy is then used by plants to perform many functions, including production of plant parts, e.g., leaves, stems and flowers. Raw materials used for this purpose are nutrients like nitrogen, phosphorus, oxygen and calcium. They are essential for growth of all plants and animals. Animals cannot perform photosynthesis. Hence, they eat plants, other animals, or dead tissue to obtain their energy and required nutrients. In ecosystems, transfer of energy and nutrients from plants to animals occurs along pathways called *'food chain'*. The first link in a food chain consists of primary producers, i.e., green plants and other organisms are capable of photosynthesis. The plant-eating organisms, called *'primary consumers'*, are the next link in the food chain. They, in turn, are eaten by the *'secondary consumers'*, i.e., carnivores (flesh eaters) or omnivores (plant and animal eaters). The *'decomposers'*, e.g., bacteria and fungi make up the final link in the food chain. They break down dead tissues and cells, providing nutrients for a new generation of producers. Most organisms in an ecosystem have more than one food source (e.g., fish feed on both insects and plants) and so belong to more than one food chain. The consequent overlapping food chains make up *'food web'*, which is a complex phenomenon with links that are constantly changing (Fig. 3-4) (Wikipedia, 2013a).

AQUATIC ANIMALS

The term *'aquatic'* theoretically applies to the animals that live in either freshwater, (called *'freshwater animals'*) or saltwater (called *'sea or marine water animals'*). The adjective *'marine'* is most commonly used for animals that live in saltwater like ocean, and sea. Invasive aquatic animals need a water habitat, but do not necessarily have to live entirely in water. On the other hand, *'aquatic animals'* are either vertebrate or

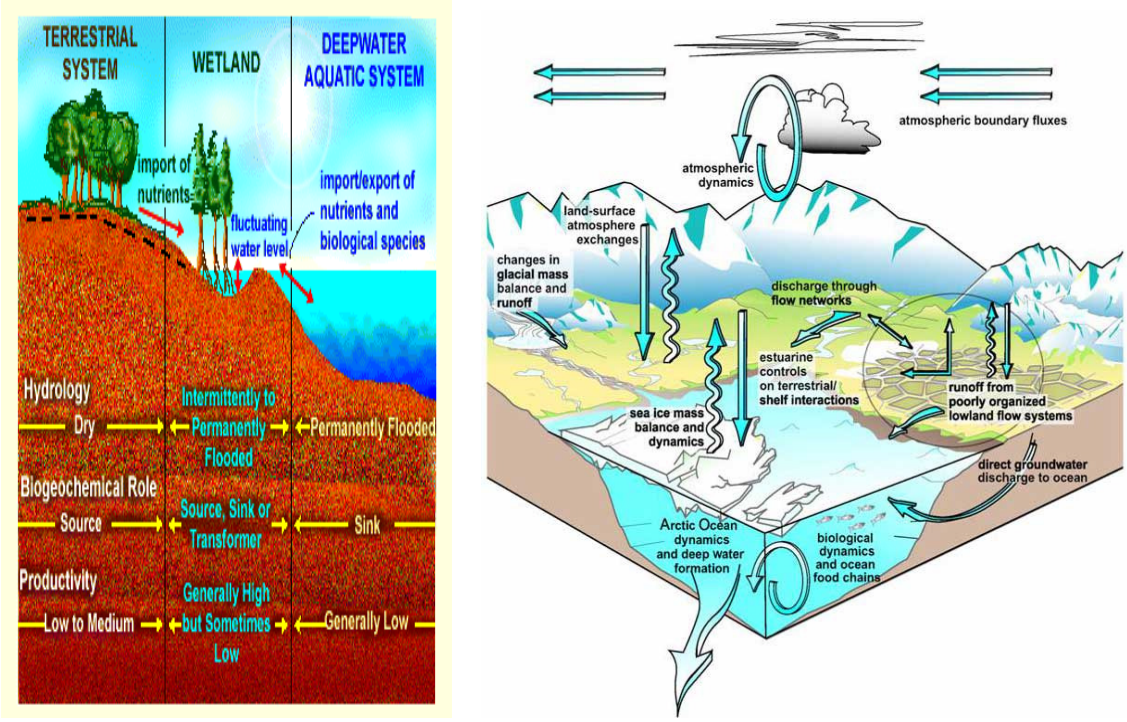

Fig. 3-4: Working of Ecosystem and Food Web

[Source of figures: Different websites which are gratefully acknowledged]

invertebrate that live in water for most or all of their lives. These animals are subject to pressure from overfishing, destructive fishing, marine pollution and climate change. The animals that move readily from water to land and *vice-versa* are known as *'amphibians'* (e.g., frog). When the animals live in water, they have special adaptations to help them survive in an aquatic habitat. The more time the animal spends in the water, the quicker they adapt to their new habitat. There are numerous ways that an aquatic animal can adapt to their habitat. The *'aquatic mammals'* are a group of aquatic vertebrates that live in the aquatic (watery) environment. Examples are: freshwater mammals like otters and *Platypus*; and marine mammals like dolphins, whales, seals, sea lions, walrus, dugong, manatees, sea otters and polar bears (Wikipedia, 2013b).

A. Vertebrate Aquatic Animals:

'Vertebrate aquatic animals' are fish that are mostly vertebrates or animals having backbones. Fish are classified into 3 main groups as per their evolutionary development-

1) *Primitive fish*- They do not have jaws and appendages, and their mouths look like

holes with armored body; e.g., lamprey fish (*Petromyzon*) and hagfish (*Myxine*).

2) *Cartilaginous fish*- They have cartilaginous endo-skeletons, and lack gill cover, instead they have gill slits; e.g., shark (*Scoliodon*) and ray (*Torpedo*) fishes.
3) *True bony fish*- In these, jaws are present and skeletons are formed by bones, with scales (e.g., *Labeo, Catla,* tuna, salmon and goldfish), or without scales (e.g., *Clarias* and *Wallago)* on their body.

B. Invertebrate Aquatic Animals:

There are a large number of invertebrate aquatic animals belonging to 9 major phyla. Certain phyla of the invertebrates are as under-

1) *Cnidarians (Cnidaria or Coelenterata)*- e.g., jellyfish, anemones, corals, hydras.
2) *Annelids (Annelida)*- They are segmented worms. The classes of this phylum are Polychaeta, Archiannelida, Oligochaeta and Hirudinea. Annelids may breathe air or extract oxygen from that dissolved in water through specialized organs known as *'gill'*, or directly through the skin. The natural environments and animals that live in them can be categorized as aquatic (water) or terrestrial (land).
3) *Arthropods (Phylum Arthropoda)*- e.g., prawn, shrimp, crab and lobster.
4) *Mollusks (Phylum Mollusca)*- e.g., *Sepia, Loligo,* oyster and *Octopus*.
5) *Echinoderms (Phylum Echinodermata)*- e.g., sea cucumbers and sea urchins. In China, sea cucumbers are farmed in artificial ponds as large as 1,000 acres.

Some arthropods and mollusks need water to breathe, while others can breathe in air. They are different classes of taxonomy and some are closely related, while others are not.

FISHES AND THEIR IMPORTANCE

The words, *'fish'* and *'fishes'* (Fig. 5-19) though often used interchangeably, have different meanings. *'Fish'* is used either as singular noun or as a group of specimens from a single species. However, *'fishes'* means a group of different species. A *'fish'* is any gill-bearing aquatic vertebrate (or craniate) animal that lacks limbs with digits. As per this definition, living hagfish, lampreys (lamper eel fish), and cartilaginous and bony fish, as well as various extinct related groups are called as *'fish'*. Because the term is defined

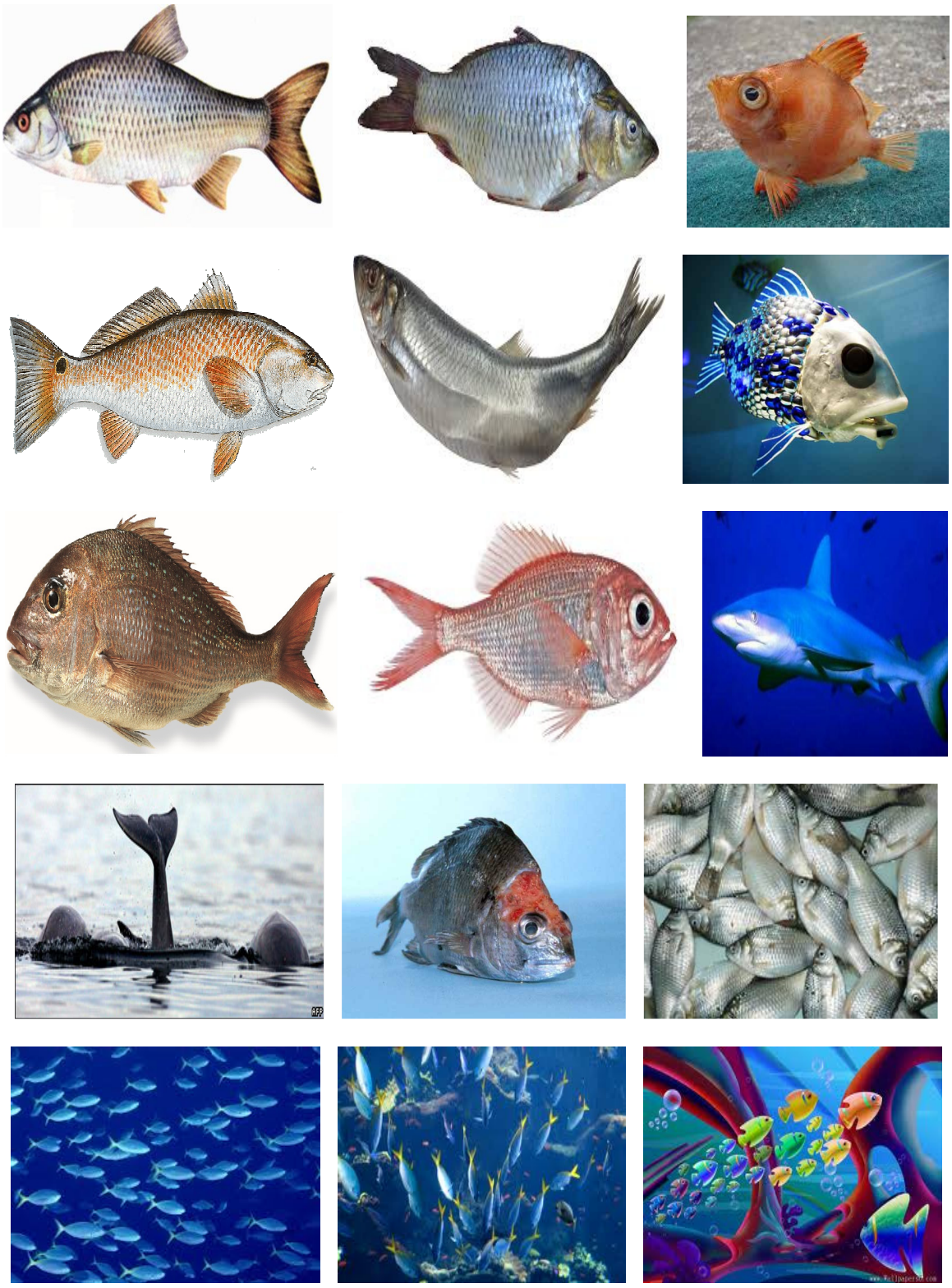

Fig. 5-19: Different Species of Fish

[Source of figures: Different websites which are gratefully acknowledged]

negatively, and excludes tetrapods (i.e., amphibians, reptiles, birds and mammals) which descend from within same ancestry, it is paraphyletic. Traditional term *'Pisces'* (ichthyes) is a typological, but not a phylogenetic classification. Unlike groupings (birds or mammals), fish are not a single clad but a paraphyletic collection of *'taxa'*, including hagfish, lamprey, shark, ray, ray-finned fish, coelacanth and lungfish. Lungfish and coelacanths are closer relatives of tetrapods (e.g., amphibians, reptiles, birds, mammals, etc.) than of other fishes such as ray-finned fish or sharks, so the last common ancestor of all fish is also an ancestor to tetrapods. As non-mammalian animals that spend their entire lives in water are *'fish'* in an informal sense; but *'finfish'* is sometimes used to distinguish *'fish'* as the animals *'defined by having vertebrae'* from *'shellfish'* (Wikipedia, 2013b).

As paraphyletic groups are no longer recognized in modern systematic biology, the use of term *'fish'* as a biological group must be avoided. So, the term *'fish'* most precisely describes any non-tetrapod craniate (i.e., animal with a skull and in most cases a backbone) that has gills and fin-shaped limbs (if any). Most fish are *'cold-blooded'*, or ectothermic, allowing their body temperatures to vary as ambient temperatures change. Fish are abundant in most water bodies; and can be found in nearly all aquatic environments, from high mountain streams (e.g., char and gudgeon) to abyssal and even hadal depths of the deepest oceans (e.g., gulpers and anglerfish). At 32,000 species, fish have greater species diversity than any other class of vertebrates (Wikipedia, 2013b).

Fish, especially as food, are an important resource worldwide. Commercial and subsistence fishers hunt fishes in wild fisheries (or farms) than in ponds or cages in the ocean. Fish are also caught by recreational fishers, kept as pets, raised by fish keepers and exhibited in public aquaria. Fish have a role in culture through the ages, serving as deities, religious symbols, and as the subjects of art, books and movies. Briefly, the importance of fish can be categorized as under (Wikipedia, 2013b):

a. Fish for incarnation of Lord Vishnu as a merman.
b. Fish as coat of arms of Narva, Estonia.
c. Fish for aquarium.
d. Economic importance of fish.
e. Recreational importance of fish.

f. Cultural and religious importance of fish- In the book, a *'great fish'* swallowed Jonah the prophet. Legends of half-human, half-fish mermaids have featured in stories. Among the deities said to take the form of a fish are *'Ika-Roa'* of Polynesians, *'Dagon'* of ancient Semitic peoples, *'Shark-Gods'* of Hawaii and *'Matsya'* of Dravidas of India. The astrological symbol *'Pisces'* is based on a constellation of the same name, but there is also a second fish constellation in the night sky, *'Piscis Austrinus'*. Fish is used figuratively in many ways, e.g., *'ichthys'* used by early Christians to identify themselves; while fish used as a symbol of fertility among Bengalis. Large fish, especially sharks, have been the subject of *'horror movies'* and *'thrillers'*, most notably the novel *'Jaws'*, which spawned a series of films of same name that in turn inspired similar films or parodies. In semiotic of *'Ashtamangala'* (Buddhist symbolism), golden fish (*'Matsya'* in Sanskrit) represents the state of fearless suspension in samsara, perceived as harmless ocean, called *'Buddha-eyes or rigpa-sight'*. Fish symbolizes auspiciousness of all living beings in a state of fearlessness without danger of drowning in *'Samsaric Ocean of Suffering'*, and migrating from teaching to teaching freely and spontaneously just as fish swim. Fish have also religious significance in Christianity, who is first signified by sign of fish, and especially referring to *'feeding the multitude'* in desert. In *'Buddhism'*, fish symbolize happiness as they have complete freedom of movement in water. They represent fertility and abundance. Often drawn in the form of carp fish, they are regarded in *'Orient as Sacred'* on account of their elegant beauty, size and life-span. The name of Canadian city of Coquitlam (British Columbia) is derived from *'Kwikwetlem'* that is derived from a *'Coast Salish'* term meaning *'little red fish'*.

Besides above, fish is also used as a model organism in different biological researches. The potential for the application of research findings to both human and environmental health issues makes fish species attractive and valuable alternative models in various diseases, including cancer, and pharmacological and toxicological research. Zebrafish has emerged as a major model organism in the developmental genetics, neurophysiology or biomedical research (Pandey, 2011; Pandey et al., 2012b).

2

POLLUTION OF AQUATIC ENVIRONMENT

AQUATIC ENVIRONMENT

'Aquatic environment' is the *'interacting system of resources'*, e.g., water and biota. The world has various lentic and lotic aquatic environments. These are main sources of food to millions of people. Abundance and distribution of fish in water are the products of interaction among fishes and their chemical, physical and biological surroundings. So, the dynamics of aquatic environment depends on the properties of water. Environmental forces like temperature, light, dissolved oxygen, current, population density which impinge on the aquatic lives are complex and inter-related in their effects. Unprecedented development on all fronts associated with fast growth of human and cattle population has caused rapid deterioration in the aquatic environment. The main effects of industrial discharge on aquatic fauna and water quality are mortality and contamination of water by *'toxic metals or pollutants'*. Thus, conservation and management of these resources with environment are of paramount interest. Exponential growth of human population and progressive industrialization pose serious threats to aquatic environment and its resources potential. To achieve better knowledge of aquatic environment, one must review wide fields of aquatic science, such as taxonomy, reproduction and growth of fish and crustaceans; planktonic organisms in aquatic bodies, mass culture and biological indicators; limnology of ponds, lakes and reservoirs; aquatic productivity and strategy to enhance it in ponds; physio-chemical nature of ponds and rivers affecting the plankton production; benthic fauna; impact of seasonal and annual rhythmic environmental changes on fish, crustaceans and molluscans, etc.; nutrient status, cycling in aquatic bodies and possible role regarding culture of fish and prawn; remote sensing applications in aquatic environment; water quality monitoring, aquatic biodiversity and wet land; management of reservoirs regarding aquaculture and water quality; and pollutions in the aquatic environment and their control measures (Wikipedia, 2013a).

ENVIRONMENTAL POLLUTION

Industrialization of the modern world has led to the proliferation of several *'pollutants or toxic metals'* in our environment. The pollutants through atmospheric pollution can accumulate in the living things any time they are taken up and stored faster than they are broken down (metabolized) or excreted. They can enter a water supply by the industrial and consumer wastes, or even from the acidic rain breaking down soils and releasing heavy metals into the streams, lakes, rivers and groundwater (Fig. 20-28). The hazardous wastes (pollutants) are poisonous byproducts of manufacturing, farming, city septic systems, construction, automotive garages, laboratories, hospitals and other industries. The waste may be liquid, solid, or sludge and contain chemicals, heavy metals, radiation, dangerous pathogens, or other toxins. Even households generate hazardous waste from items like batteries, used computer equipment and leftover paints or pesticides. All these can harm humans, animals, aquatic organisms and plants if they encounter these toxins buried in ground, stream runoff, groundwater that supplies drinking water, or in floodwaters. Some *'toxicants/pollutants'* such as mercury persist in the environment and accumulate. Humans or animals often absorb them when they eat fish. Mercury in water can cause abnormal behaviour, slower growth and development, reduced reproduction, and death of aquatic animals. Too much sodium chloride (ordinary salt) in water may kill aquatic animals/fish. Many times, the heavy metals are included in the fertilizers to be used by the farmers on food crops, or sold directly to the public. However, the dangerous levels of such metals are taken up by some plants and subsequently eaten by people, with particularly negative effects on children. *'Persistent organic pollutants'* may cause declines, deformities and death of fish. *'Nutrient pollutions'* (e.g., nitrogen, phosphates, etc.) causes overgrowth of toxic algae eaten by several kinds of aquatic organisms and may lead to their death. The nutrient pollutions can also cause outbreaks of fish diseases. '*Oil pollution'* (as part of chemical contamination) can negatively affect the development of aquatic organisms. Fish may be polluted with oil, having increased susceptibility to disease. The oil pollution can affect reproductive process, and may also cause gastrointestinal irritation and damage to liver, kidney and nervous system (Pandey, 2013a).

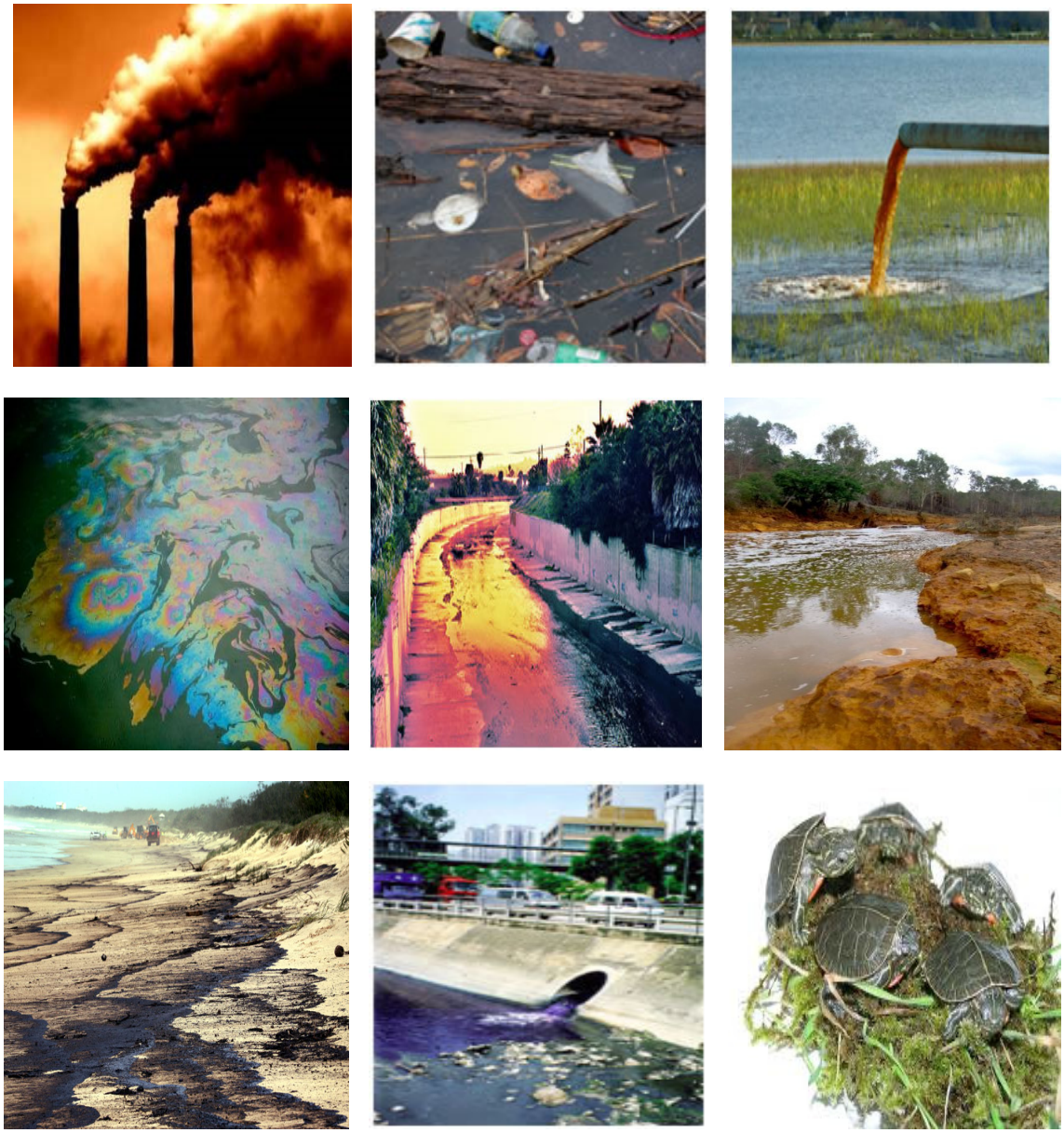

Fig. 20-28: Different Forms of Pollution to Aquatic environment
[Source of figures: Different websites which are gratefully acknowledged]

POLLUTION OF WATER

'Water pollution' occurs when a body of water is adversely affected due to addition of large amounts of materials into water, as described below (Water Pollution, 2006).

Types of Water Pollution:

1. ***Toxic or chemical water pollution-*** A *'toxic substance'* is a chemical pollutant which

is not a naturally occurring substance in aquatic ecosystem. Toxic pollution in water greatly occurs due to adding of pesticides (herbicides) and industrial compounds.

2. ***Organic water pollution-*** It occurs when an excess of organic matter, e.g., manure or sewage, enters the water. When organic matter increases in a pond, the number of *'decomposers'* will increase. These decomposers grow rapidly and largely use oxygen during their growth. This leads to a depletion of oxygen as the *'decomposition process'* occurs. A lack of oxygen can kill the aquatic organisms. As the aquatic organisms die, they are broken down by decomposers that lead to further depletion of oxygen levels. Another form of organic pollution may occur when inorganic pollutants like nitrogen and phosphates accumulate in the aquatic ecosystem. These nutrients in high amounts cause an overgrowth of plants and algae. When these plants and algae die, they become organic material. The enormous decay of these matters then lowers oxygen level. This process of rapid plant growth, followed by increased activity by decomposers and a depletion of oxygen level is called *'eutrophication'*.
3. ***Thermal water pollution-*** It can occur when water is used as a coolant near a power or industrial plant, and then is returned to aquatic environment at a higher temperature than it was originally. The thermal pollution can lead to a decrease in dissolved oxygen level in the water, while also increasing the biological demand of aquatic organisms for oxygen.
4. ***Ecological water pollution-*** It occurs when the chemical, organic and thermal water pollutions are caused by nature rather than by human activity. For instance, an increased rate of siltation of a waterway after a landslide would increase the amount of sediments in runoff water. Similarly, when a large animal like a deer drowns in a flood, so large amount of organic material is added to water. Major geological events (e.g., a volcano eruption) might also be sources of ecological water pollution.

Sources of Water Pollution:

The sources of water pollution may be *'point source'* or *'non-point source'*. The *'point source'* of pollution occurs when any polluting substance is emitted directly into the waterway, e.g., a pipe spewing toxic chemicals directly into a river. However, the

'non-point source' occurs when there is runoff of pollutants into a waterway, e.g., when fertilizer from a field is carried into a stream by surface runoff. The specific sources of water pollution are discussed below (Water Pollution, 2006).

1. ***Homes-***
   a) Fertilizers and pesticides (or herbicides) used for lawn care can runoff and contaminate the waterway. As with agricultural fertilizers, home fertilizers can lead to eutrophication of rivers and lakes.
   b) Sewage generated by houses or runoff from septic tanks into nearby waterways, introduce organic pollutants which can cause eutrophication.
   c) Leakage of oil and antifreeze from a car on a driveway can be washed off by the rain into nearby waterways, polluting it.
   d) Improper disposal of hazardous chemicals down the drain introduce toxic materials into the ecosystem, contaminating the water supplies in a way that can harm aquatic organisms.
2. ***Farming-***
   a) Million tons of soils are carried by many rivers to the gulf and oceans each year. This siltation is largely due to runoff from exposed agricultural soils. Excessive amounts of sediment in waterways can block sunlight, preventing aquatic plants from photosynthesizing, and can suffocate fish by clogging their gills.
   b) Allowing livestock to graze near water sources often cause organic waste products being washed into waterways. This sudden adding of organic material increases the amount of nitrogen in water, and can also lead to eutrophication.
   c) Farms often use large amounts of pesticides, which are toxic pollutants. These chemicals are particularly dangerous to life in rivers, streams and lakes, where toxic substances can build up over a period of time.
   d) Farms also frequently use large amounts of fertilizers, which are washed into the waterways, and damage the water supply and aquatic lives. The fertilizers can increase the levels of nitrates and phosphates in water, leading to eutrophication.
3. ***Business-***
   a) Many industrial and power plants use rivers, streams and lakes to dispose of waste

heat. The resulting hot water can cause thermal water pollution, which may have a disastrous effect on the life in an aquatic ecosystem as the increased temperature decreases oxygen levels in water, thereby reducing the aquatic animals.

b) Waste and sewage generated by industry can get into the water supply, introducing large organic pollutants into the ecosystem.
c) Water can become contaminated with toxic or radioactive materials from industry, mine sites and abandoned hazardous waste sites.
d) *'Acid precipitation'* is caused when the burning of fossil fuels emits sulphur dioxide (SO_2) into the atmosphere. Sulphur dioxide reacts with water in the atmosphere, creating rainfall, which contains sulphuric acid. As acid precipitation falls into lakes, streams and ponds, it can lower the overall pH of waterway and can kill the vital plant lives, thereby affecting the whole food chain. It can also leach heavy metals from the soil into water, killing fish and other aquatic organisms. Because of this, air pollution is potentially one of the most threatening forms of pollution to aquatic ecosystems.
e) Clearing of land can cause erosion of soil into the river.

POLLUTANTS OF AQUATIC ENVIRONMENT

A *'pollutant or hazardous substance'* is one which can cause harm to the environment or life. Most of such substances are synthetic, e.g., pesticides (including herbicides), polychlorinated biphenyls (PCBs), dioxins and furans (Wikipedia, 2013a). The potential for accumulation of hazardous substances (*'toxicants/toxic substances/ pollutants/contaminants'*) within tissues increases the significance of certain pollutants which may be present in water even though the ambient concentrations are very low. The biota of flowing waters may be restored, following catastrophic entry of pollutants, by drift from unaffected regions upstream. The range of potential hazardous or toxic substances is very extensive and includes inorganic poisons, organic poisons, heavy metals, PCBs and pesticides. The metals, PCBs and pesticides have the greatest potential for bioaccumulation (Hellawell, 1988; Pandey et al., 2013).

Whilst the synthetic chemicals are important to the society, some of them are

'hazardous (toxic)', raising concerns for body health and environment depending on their pattern of use and the potential for exposure. Certain types of naturally occurring chemicals, e.g., metals, can also be hazardous. These hazardous substances comprise a wide range of industrial and household chemicals, metals, pesticides and pharmaceuticals. The data show that the hazardous substances, such as DDT, hexchlorobenzene (HCB), lindane and PCBs can be found at high concentrations in the aquatic environment. Some hazardous substances, e.g., mercury, tributyltin and polyaromatic hydrocarbons (PAHs) are hydrophobic and tend to accumulate in sediment and biota, with the result that their concentrations are likely to be higher. The *'emerging pollutants'* include the substances which have existed for some time, e.g., pharmaceuticals and personal care products, but also relatively new substances, e.g., nanomaterials (EEA, 2011; Pandey et al., 2013).

Several studies identified the toxic or hazardous substances as potentially harmful to the aquatic lives in marine environment. In Europe, the *'Environmental Quality Standards (EQSs) Directive'* has been adopted for *'dangerous or hazardous substances'* to control pollution caused by certain dangerous substances discharged into the environment. This has established two lists of compounds: (a) *'List-I'* (Table 1) deals with the substances, which are particularly dangerous because of their toxicity, persistence and bioaccumulation. Thus, the pollution by the *'List-I'* substances must be eliminated; and (b) *'List-II'* (Table 2) contains the substances which are less dangerous but they nevertheless have a deleterious effects on the aquatic environment. Therefore, the pollution by the *'List-II'* substances must be reduced. Several other substances have also been reported which are dangerous and can cause pollution in the aquatic environment (Table 3) (UKMSAC, 2013).

Table 1: List-I Substances Highly Dangerous to Aquatic Environment

Aldrin	1,2-dichloroethane	Isodrin	Trichlorobenzene
Cadmium	DDT	Lindane (hexachlorocyclohexane)	Trichloroethylene
Chloroform	Endrin	Mercury	
Carbon tetrachloride	Hexachlorobenzene	Pentachlorophenol	
Dieldrin	Hexachlorobutadiene	Perchloroethylene	

Table 2: List-II Substances Less Dangerous to Aquatic Environment

Arsenic	4-chloro-3-methyl phenol	Dichlorvos	Mecoprop	Toluene
Atrazine	Chloronitrotoluene	Dimethoate	Mothproofer	Triazophos
Azinphos-methyl	2-chlorophenol	Endosulphan	Naphthalene	Trichloroethane
Benzene	Chromium	Fenitrothion	Nickel	Trifluralin
Bentazone	Copper	Iron	Omethoate	Vanadium
Biphenyl	Demeton	Lead	Organotin	Xylene
Boron	2,4-dichlorophenol	Linuron	Simazine	Zinc

Table 3: Other Pollutants of Aquatic Environment

Abamectin	Chlorotoluron	Fluoride	Manganese	Pirimicarb
Aluminium	Chlorpropham	Flusilazole	Mevinphos	Pirimiphos methyl
Ammonia	Chromium	Formaldehyde	Nonylphenol	PAHs
Brominated flame retardants	Cobalt	Imazethypyr	Nonylphenol ethoxylate	Polybrominated naphthalenes
Bromine	Cyanide	Inorganictin	NTA	PCBs and PCB substitutes
Bromoxynil	Dichlorobenzene	Ioxynil	Octylphenol	Prochloraz
Carbendazim	Dichlorphen	Isoproturon	Oxolinic acid	Propyzamide
Chlorinated paraffin	Diflubenzuron	Ivermectin	Oxytetracycline	Silver
Chlorine	Doramectin	Macozeb	Pendimethalin	Styrene
Chlorine dioxide	EDTA	Malachite green	Phenol	Timber treatment chemicals
Chlorothalonil	Ethofumesate	Maneb	Phthlates	Triazine

TOXICITY OF POLLUTION ON AQUATIC ENVIRONMENT

The toxic effects of *'pollution'* on the whole organism can be of three main categories: neurophysiological, behavioural and reproductive effects. These effects can often be inter-related: neurological changes can affect behaviour; changes in behaviour can affect reproduction and so on. A compound does not always have the effect on a target organism or a community. It always depends on the concentration of that compound and the time of exposure to it. These effects eventually can be either *'acute'* or *'chronic'*. The *'acute toxicity'* occurs rapidly, and is often fatal and rarely reversible. However, the *'chronic toxicity'* develops after long exposure to low doses or it has long effect after exposure and may ultimately cause death. A *'poison'* is lethal when it causes death, or sufficient to cause it, by direct action; and a *'poison'* is sublethal when it is below the level which directly causes death. Then it results in the regression of physiological or behavioural processes of the organism, and its overall fitness is reduced.

Only in the case of radioactive pollution, it is likely that it will cause irreversible effects on the ecosystem (environment). The toxic effects of pollution on freshwater species cause the loss of some species. Generally, there may be a reduction in diversity and a change in the balance of such processes as predation, competition and materials cycling. Because of the complexity of pollution, the effects of take-up in the aquatic life are also dependent on the pollutants characteristic feature. If two or more poisons are present together in an effluent, they may exert a combined effect to an organism, which can be additive, antagonistic or synergistic. An example of an additive interaction is the combined toxicity of cadmium and zinc to fish. Calcium is antagonistic to lead, aluminium and zinc. Copper is more than additive with chlorine, zinc, cadmium and mercury, while its decreases the toxicity of cyanide. The toxicity to the mayfly (*Baetis rhodani*) of ammonia and phenol at low concentrations is additive, but at higher concentrations the effect is more than additive. The fate of wastes (or pollutants) in the aquatic environment and their effects on the organisms, population and community are shown in Figure 29 (Lenntech, 2013).

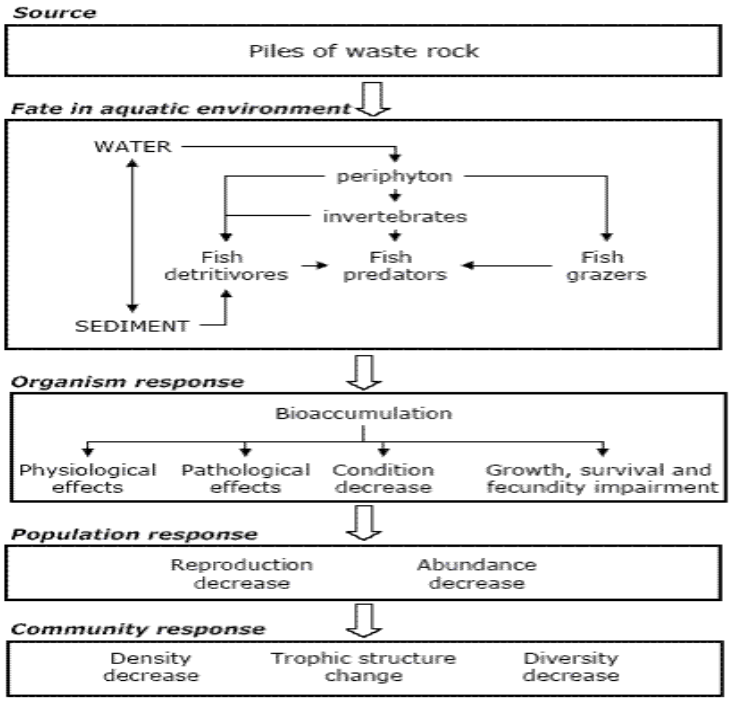

Fig. 29: Fate of Pollutants and their Effects on Aquatic Environment
[Source of figure: Lenntech website which is gratefully acknowledged]

3

HAZARDS OF TOXIC SUBSTANCES TO AQUATIC ENVIRONMENT

EMISSION OF TOXIC SUBSTANCES TO AQUATIC ENVIRONMENT

'Toxic or hazardous substances' are emitted to water bodies both directly and indirectly through diffuse and point sources from a wide range of land-based and marine sources, including agriculture and aquaculture, industry, oil exploration, mining activities, transport, shipping and waste disposal, as well as our own homes. The emission of hazardous substances to the environment can occur at every stage of their life cycle, from production, processing, manufacturing and use in downstream production sectors or by the general public to their eventual disposal. The sources include industrial activities of course, but also the urban environment, agriculture, mining, landfills and contaminated areas. Besides, chemical contamination arising from the exploitation of shale gas may also cause the emission of hazardous substances. In regions where more intense rainfall is expected, the frequency and severity of polluted urban storm flow can be increased; whilst the flushing to water of agricultural pollutants, including pesticides and veterinary medicines may be exacerbated. Hotter, drier summers and increasingly severe and frequent droughts will deplete river flows, reducing contaminant dilution capacity and leading to elevated concentrations of hazardous substances. Rising water temperatures and other stressors associated with climate change may interact with hazardous substances to impact the immune system health of aquatic organisms. Ocean acidification driven by increasing atmospheric carbon dioxide (CO_2 in water becomes carbonic acid) may change the speciation of metals in seawater and, therefore, their interaction with marine organisms. In addition, coastal erosion (likely to be also intensified by climate change) may lead to the exposure of historical landfill sites along the coastlines, releasing hazardous substances to their coastal waters (EEA, 2011).

TOXIC EFFECTS OF UNHEALTHY AQUATIC ECOSYSTEM

'Healthy aquatic ecosystem' is that where human disturbances have not impaired the natural functioning (e.g., nutrient cycling), nor appreciably altered the structure (e.g., species composition) of system. However, an *'unhealthy aquatic ecosystem'* is one where the natural state is out of balance. These disturbances can be *'physical'* (e.g., injection of abnormally hot water into a stream), *'chemical'* (e.g., introduction of toxic wastes at concentrations harmful to organisms), or *'biological'* (e.g., introduction and propagation of non-native animal or plant species). Different processes of accumulation of toxic substances in the aquatic environment have been described in Figures 30 to 36. Some toxic substances can enter a *'food web'* and be transferred through it. The uptake of any environmental substance by an organism is called *'bioconcentration'*. Although nutrients taken up through this process are usually converted into the proteins or excreted as waste, many toxic compounds accumulate in the fat or certain organs (e.g., liver) of animals. As contaminated organisms are eaten by others, the toxic substances are transferred up the levels in food web and become more concentrated, sometimes to harmful levels. This process is called *'biomagnification'*. The species at the top level of food web, including humans, are often subjected to higher concentrations of toxic substances than those at the bottom. So, toxic substance reaching harmful level is one sign that the aquatic ecosystem is unhealthy. When an ecosystem is out of balance, the humans and animals will begin to suffer. The health and many activities of humans and animals are dependent on the health of aquatic ecosystems. Most of the water that we drink is taken from lakes or rivers. If the lake or river system is unhealthy, the water may be unsafe to drink or unsuitable for industry, agriculture, or recreation even after treatment (Wikipedia, 2013a).

The effects of toxicants in flowing waters are modified by unidirectional transport and dispersion which afford the potential for a degree of *'self-purification'*. The chemical quality of receiving water also affects toxicity. Biological factors also contribute to the ultimate effect of pollutants. Few generalizations can be made regarding the effects of toxic substances on the biota. Each species tends to respond to different toxicants in different ways and even at different stages in its life-history (Hellawell, 1988).

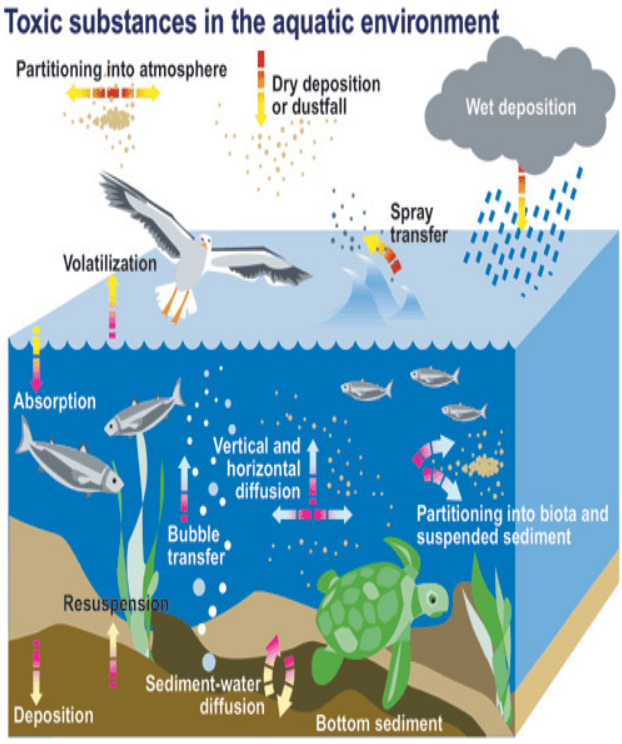

Fig. 30

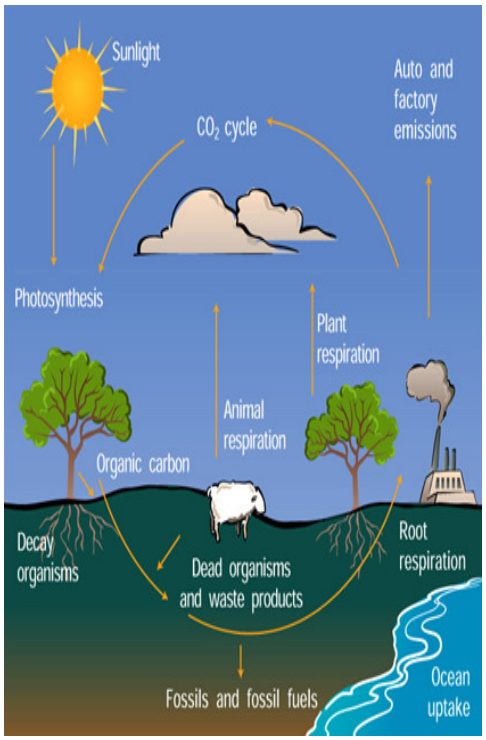

Fig. 31

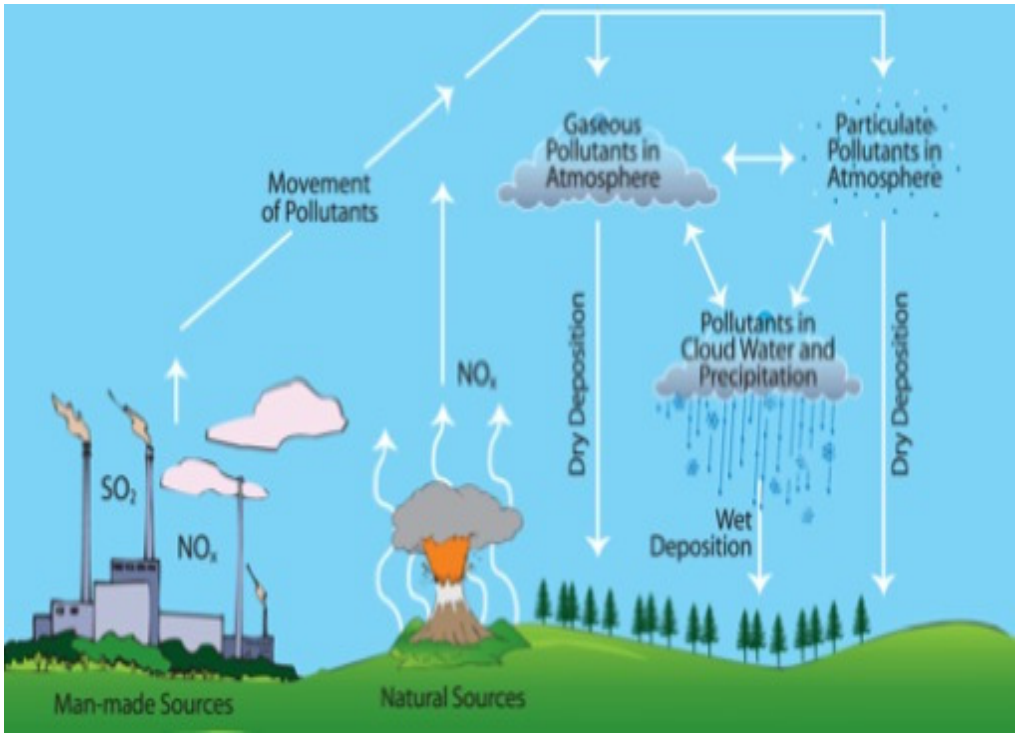

Fig. 32

Fig. 30-32: Pollution and Cycle of Toxic Substances in Aquatic Environment

[Source of figures: Different websites which are gratefully acknowledged]

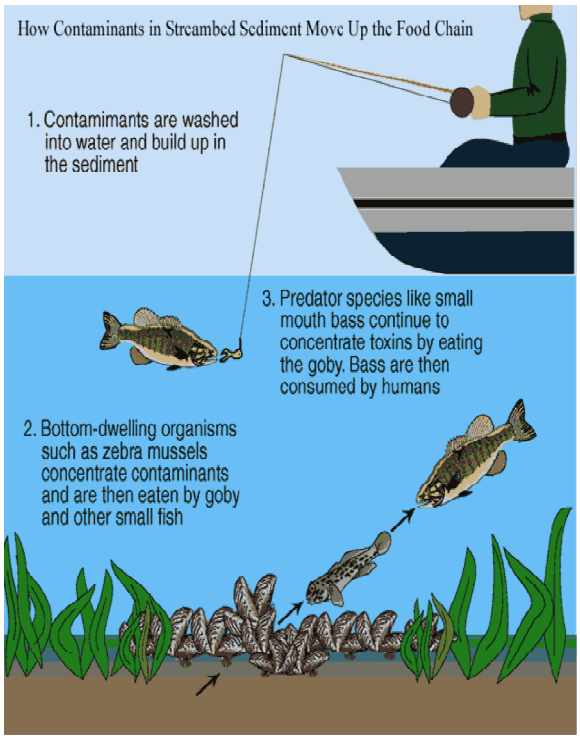

Fig. 33

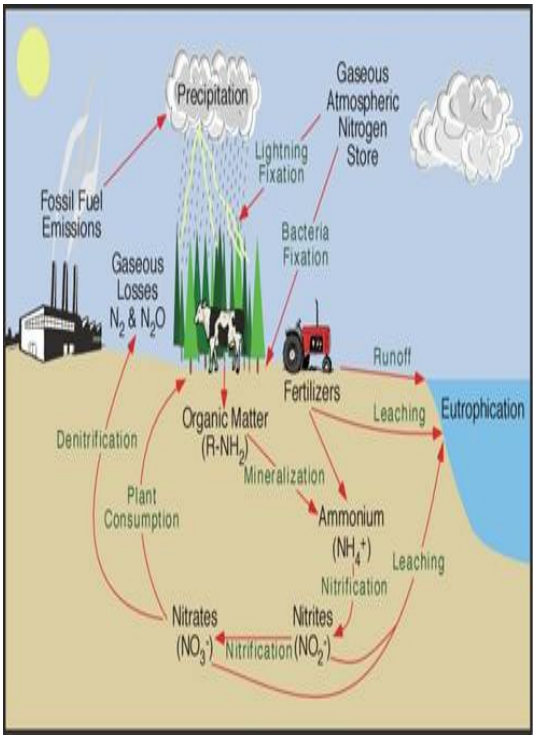

Fig. 34

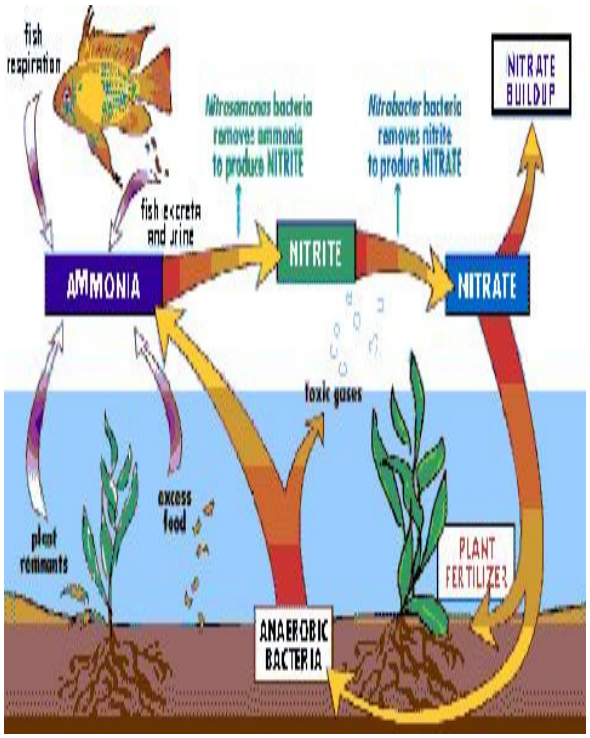

Fig. 35

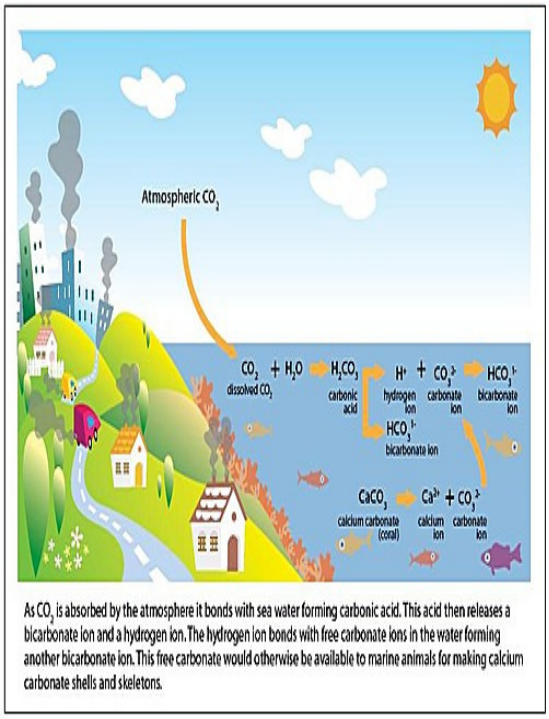

Fig. 36

Fig. 33-36: Pollution and Cycle of Toxic Substances in Aquatic Environment

[Source of figures: Different websites which are gratefully acknowledged]

The uses of aquatic ecosystems are impaired when these systems are unhealthy, for instances (Wikipedia, 2013a):

- Navigation problems for pleasure craft due to rapid expansion of bottom-rooted aquatic plants have increased.
- Proliferation of non-native species has created problems, e.g., rapidly expanding zebra mussel population introduced from ballast waters of a European freighter into the Great Lakes. The zebra mussels have few natural predators and because a female can produce 30,000 eggs yearly, they may spread into most of freshwater systems. This species is clogging industrial and municipal water treatment intake pipes, coating boats and piers, and causing beach closures.
- The inland and coastal commercial fisheries have been shut down due to fish or shellfish contamination or the loss of an important species from the ecosystem.
- Frequency of urban beach closures has escalated as a result of contamination by animal faeces and medical waste.

The *'unhealthy or toxic aquatic ecosystem'* (or *'poor ecosystem health'*) may cause:

- Accelerated proliferation of organisms, e.g., algae blooms produced by excessive phosphorous and nitrogen in water. This is called *'eutrophication'* (Fig. 34).
- Change in chemical properties, mostly due to reduced pH in water by acid rain.
- Presence of certain organisms that indicate unsanitary conditions, e.g., coliform bacteria are a sign which the system may contain organisms that produce various human diseases like diarrhoea, typhoid and cholera.
- Increased incidence of tumours or deformities in animals.
- Many other symptoms, e.g., increased lake acidity, which may kill certain species, thereby allowing the temporary proliferation of species more tolerant of acidity.
- Loss of traditional aboriginal culture associated with ecosystem.
- Loss of species (Wikipedia, 2013a).

Hazardous substances (pollutants) have detrimental effects on aquatic biota at molecular, cellular, tissue, organ and ecosystem level. For example, the substances with endocrine disrupting properties have been shown to impair the reproduction in fish and shellfish, raising concerns for fertility and population survival. Impact of organochlorines

upon sea birds and marine mammals is also well noted, as is the toxicity of metals and pesticides to freshwater biota. These toxic effects also diminish the profit given by aquatic ecosystems, and consequently the revenue which can be obtained from them. In humans, the man-made chemicals have caused a range of chronic diseases like cancer, as well as reproductive and developmental impairment. Such exposure can be linked to the presence of hazardous substances in water, through ingestion of contaminated drinking water and consumption of contaminated freshwater fish and seafood. Laboratory studies have shown that the combined effects of chemicals upon aquatic life can be additive resulting in detrimental effects even if the chemicals are present, individually, at levels below which any adverse effects can be detected (EEA, 2011; Pandey et al., 2013).

Industrialization may produce high concentrations of toxic substances in water, affecting freshwater ecosystems with acute and chronic health effects. The toxic substances can significantly damage certain physiological and biochemical processes when they enter the organs of fish and other aquatic organisms. The liver is an important organ involved in metabolic processes and in detoxification of xenobiotics. In some conditions, the toxic substances can accumulate in liver to toxic levels and cause pathology. To prove this evidence, acute toxicity of antimony (Sb) heavy metal and its effects at sublethal concentrations on the oxygen consumption and hepato-pancreas were studied in freshwater swamp shrimp (*Macrobrachium nipponense*). The 96-hr median lethal concentration (LC_{50}) value was found to be 6.748 (5.728-7.950) mg Sb/L for adult *M. nipponense* and 1.635 (1.271-2.103) mg Sb/L for juvenile *M. nipponense*. The juvenile *M. nipponense* were exposed to four different sublethal levels of antimony (0.1, 0.4, 0.8, and 1.2 mg Sb/L) over a 7-day test period and a 7-day recovery period. After 30 minutes (acute), there was an increase in the amount of oxygen consumed in all exposed groups. On days 3, 7 and 14, the decreases in oxygen consumption were significant ($p<0.05$) for the higher-exposure level groups (0.8 and 1.2 mg Sb/L). The microscopic examination showed histopathological changes such as lumen degeneration, lumen volume reduction and epithelial cell damage in the hepato-pancreas. The results indicated that antimony is a potential pollutant, causing hazardous effects in the aquatic environments (Yang et al., 2010).

4

CONCENTRATIONS OF HEAVY METALS IN FISH

INTRODUCTION TO HEAVY METALS

'Heavy metal' refers to any metallic chemical element which has a relatively high density, and is *'toxic or poisonous'* to humans and animals including fish at low concentrations, e.g., arsenic (As), lead (Pb), mercury (Hg), cadmium (Cd), chromium (Cr), thallium (Tl), etc. The heavy metals are natural components of the earth's crust. They cannot be degraded or destroyed. To a small extent, they enter our bodies via food, drinking water and air. In the form of *'trace elements'*, some heavy metals, e.g., copper (Cu) and zinc (Zn) are essential to maintain the metabolism of body. However, at higher concentrations, they can cause *'poisoning'* (Lenntech, 2013; Pandey, 2013a). Heavy metals are metallic chemical elements which have a relatively high density, and are toxic at low concentrations. Excessive concentrations of heavy metals in biological systems are detrimental to body health. Thus, the *'toxic heavy metals'* (Fig. 37-42) destabilize ecosystems because of their bioaccumulation in organisms, and produce toxic effects on the biota and even death of the most living organisms (Gupta, 2013; Pandey, 2013a).

A heavy metal is any one of the number of elements that exhibit metallic properties. These may be transition metals, lanthanides, actinides, as well as metalloids (e.g., arsenic and antimony). Typically, the term *'heavy metal'* refers to elements of atomic number 21 or higher (e.g., scandium). Some heavy metals (e.g., copper, iron, chromium and zinc) are essential in very low concentrations for survival of lives. These are called as *'essential trace elements'*. However, when they are present in greater quantities such as lead, cadmium and mercury, which are already toxic in very low concentrations, they can cause metabolic anomalies or toxicity (Heavy Metals, 2013; Pandey, 2013a).

The dictionary definition of heavy metal is *'an element with a specific gravity greater than 5'*. Other mineral elements of nutritional significance which can also be included under the term *'heavy metal'* are vanadium, cobalt, copper, iron, manganese,

molybdenum (Mo), zinc and chromium (Henry and Miles, 2001). The usage of the term *'heavy metals'* has centered on the treatment of certain metallic elements, which have an impact upon plants or animals when discharged into the natural environment in relatively high concentrations. Main heavy metals related to the environmental science are lead, cadmium, mercury, copper, chromium, zinc, manganese (Mn), nickel (Ni) and silver (Ag), etc. The heavy metals are generally all of the transition elements, e.g., those elements with an incomplete d-shell (Heavy Metals, 2013; Pandey, 2013a).

Fig. 37

Fig. 38

Fig. 39

Fig. 40

Fig. 41

Fig. 42

Certain Toxic Heavy Metals- Fig. 37: Different Heavy Metals; Fig. 38: Lead Chloride; Fig. 39: Mercuric Chloride; Fig. 40: Pentahydrate Copper Sulfate; Fig. 41: Zinc Sulphate; Fig. 42: Manganese Oxide-Hydroxide

[Source of figures: Different websites which are gratefully acknowledged]

TOXIC CONCENTRATIONS OF HEAVY METALS IN ECOSYSTEM AND FISH

As regard to heavy metals in *'marine ecosystem'*, they enter the sea normally through riverine influx (after weathering and erosion of rocks), anthropogenic activities and atmospheric deposition (dust particles, e.g., from volcano's). Human beings add both to

the riverine deposition (waste water of factories) and atmospheric deposition (cars, factories, etc.). Heavy metals are stable and cannot be broken down, which makes easy for them to accumulate in the environment. The heavy metals introduced in the marine ecosystem are mostly concentrated in the coastal areas, near densely populated and industrialized regions. They are generally associated to particles. These particles are often very small and may, therefore, stay in solution for a very long time. Nevertheless they end up in the sediments, so their concentrations in the sediments are often 10 to 100 times higher than those in the solution. In the sediments, these particles may form an important secondary source of contamination, even after the primary source has disappeared. When present above their threshold concentrations, all heavy metals are toxic. The threshold concentration depends on the specific metal and animal species but also on the environment, which determines the availability. The exposure of marine or aquatic organisms to toxic levels of metal contaminants can cause damage to tissue, inability to regenerate damaged tissue, growth inhibition and damage to DNA. Although most marine or aquatic organisms tend to accumulate heavy metals from the environment, they are capable to store, remove (through faeces, eggs or molting) or detoxify (with metallothioneins) many heavy metals. However, these abilities tend to differ between species, making some species more tolerant than others. Although detoxifying mechanisms exist but when the environmental concentrations of heavy metals are high, these mechanisms are insufficient and the marine or aquatic organisms will start showing toxic effects (Heavy Metals in Aquatic System, 2013).

All organisms, from bacteria and yeast to humans, respond to physical and chemical stressors by increasing the synthesis of a small group of *'cellular stress proteins'*. The polyacrylamide gel electrophoresis was used to detect stressor-induced, concentration-dependent changes in cellular stress protein levels in two fish cell culture systems, whereas simultaneous *in vitro* neutral red uptake cytotoxicity assays measured the stressor's effect on cellular physiology. There was a direct concentration-dependent relationship between sublethal cytotoxic effects and the increases in stress protein levels. Increases of 50 to 200% were detected in stress proteins from desert topminnow, *Poeciliopsis lucida* hepatoma-derived cell cultures exposed to cadmium (6 proteins) or

copper (4 proteins). Three proteins showed similar increases in winter flounder, *Pleuronectes americanus* kidney cell cultures exposed to the same stressors. Increases in evolutionarily conserved heat-shock protein hsp70 were detected in each experiment; its level increased with increasing stressor concentrations. In another study, 200 fish samples were collected seasonally and the concentrations of lead, cadmium, copper, zinc and iron were measured in muscle, gill, liver and gonad of three fish species (*Sparus aurata*, *Dicentrarchus labrax* and *Mugil cephalus*). The concentrations of these heavy metals were determined by using *'flame atomic absorption spectrophotometer'* (FLAAS) and *'graphite furnace atomic absorption spectrophotometer'* (GFAAS) after wet digestion method. The results indicated that all these heavy metals were found highest in muscle tissue of *S. aurata*. Although cadmium and zinc were found in spring, the iron, copper and lead were found in winter season. In addition, *D. labrax* and *M. cephalus* accumulated lowest of these heavy metals in muscle. While the highest concentrations of iron, copper and zinc were measured in the liver of *M. cephalus,* the highest levels of cadmium and lead were determined in gill tissue of *M. cephalus.* However, in some seasons, the concentrations of cadmium, lead and zinc in the muscle were higher than the maximum levels set by the law. For each fish species in spring high levels of zinc; for *D. labrax* and *M. cephalus* in spring and for *S. aurata* in winter high levels of lead; and for *S. aurata* in spring and for *M. cephalus* in winter high levels of cadmium were measured for human consumption (Heavy Metals Concentration in Fish, 2013).

Further, the concentrations of lead, cadmium, copper, zinc, iron and chromium were estimated in muscle, gill and liver of 6 fish species (viz., *Sparus auratus*, *Atherina hepsetus*, *Mugil cephalus*, *Trigla cuculus*, *Sardina pilchardus* and *Scomberesox saurus*), and relationships between fish size (length and weight) and metal concentrations in the tissues were measured by linear regression analysis. The metal concentrations (as µg/g body weight) were highest in the liver, except for iron in gill of *Scomberesox saurus* and lowest in muscle of all fish species. The highest concentrations of cadmium (4.5), chromium (17.1) and lead (41.2) were measured in the liver tissues of *T. cuculus*, *Sardina pilchardus* and *A. hepsetus*, respectively. The liver of *M. cephalus* showed strikingly high copper concentrations (202.8). The gill of *Scomberesox saurus* was the only tissue that

showed highest (885.5) iron concentration. Results of linear regression analysis showed that, except in a few cases, significant relationships between metal concentrations and fish size were negative. Highly significant ($P<0.001$) negative relationships were found between fish length and chromium concentrations in the liver of *A. hepsetus* and *M. cephalus*, and chromium concentrations in gill of *T. cuculus*. Chromium and lead concentrations in liver and copper concentrations of all tissues of *Scomberesox saurus* also showed very significant ($P<0.001$) negative relationships. In one more experiment, the concentrations of lead (Pb), cadmium (Cd), copper (Cu), arsenic (As) and zinc (Zn) in kidney, liver, gill and heart of African catfish (*Clarias gariepinus*) were estimated using *'bulk scientific atomic absorption spectrophotometer'* (BSAAS). The concentration of superoxide dismutase (SOD), catalase (CAT), glutathione S-transferase (GST) and glutathione (GSH), and formation of malondialdehyde (MDA) were estimated. The accumulation trend of these metals in the organs was: heart- Zn>Cu>Pb>As>Cd; gill- Zn>Cu>Pb>Cd>As; kidney- Zn>Cu>Pb>As>Cd; and liver- Zn>Cu >Pb>As>Cd. The order of concentration was: As- kidney>liver>gill>heart; Zn- gill>liver>kidney>heart; Pb- liver>kidney>gill>heart; Cu- kidney>liver>gill>heart; and Cd- liver>gill>kidney> heart. The levels ranged between 0.25 and 8.96 ppm in heart; 0.69 and 19.05 ppm in kidney; 2.10 and 19.75 ppm in liver; and 1.95 and 20.35 ppm in gill. The SOD activity increased by 61% in liver, 50% in the kidney and 28% in heart; while a significant ($P<0.001$) decrease (44%) was observed in the gill of *Cl. Gariepinus*i. On contrary, there was 46%, 41%, 50% and 19% decrease in CAT activity of liver, kidney, gill and heart, respectively. The levels of GST activities of liver, kidney and heart increased ($P<0.001$) by 62%, 72% and 37%, respectively. The GSH concentration increased by 81%, 83% and 53% in liver, kidney and heart, respectively but decreased by 44% in gill. The MDA levels were significantly ($P<0.001$) elevated in liver, kidney, gill and heart by 177%, 102%, 168% and 71%, respectively. Overall, the results indicated that alteration in antioxidant enzymes, glutathione system and induction of lipid peroxidation reflects the presence of heavy metals, which may cause oxidative stress in *Clarias gariepinus*. The study, therefore, provides a rational use of biomarkers of oxidative stress in biomonitoring of aquatic pollution (Heavy Metals Concentration in Fish, 2013).

In a study (Vinodhini and Narayanan, 2008), *Cyprinus carpio* (common carp) fishes were exposed to cadmium (Cd), chromium (Cr), nickel (Ni) and lead (Pb) at sublethal concentrations for periods of 32 days. Accumulation of heavy metal gradually increased in the liver during heavy metal exposure period. The order of heavy metal accumulation in the gills and liver was Cd>Pb>Ni>Cr and Pb>Cd>Ni>Cr, respectively. Similarly, in case of kidney and flesh tissues, the order was Pb>Cd>Cr>Ni and Pb>Cr>Cd>Ni, respectively. In all heavy metals, the bioaccumulation of lead and cadmium proportion was significantly increased in the tissues of *C. carpio*.

BIOMARKER OR EVALUATION OF AQUATIC HEAVY METALS

The evaluation of water quality standards for heavy metals has rapidly increased worldwide, and still there is a great need for evaluation of water quality standards. In both laboratory and field studies, it is important that the weaknesses and limitations of available techniques are recognized. The status of water quality standards on the basis of toxicity data, field observations and use of environmental standards for effluent control, all these should be studied. *'Toxicity tests'* should be reviewed and their common failings be identified. The scientifically accepted data should be reviewed from time to time for individual metals for freshwater fish, invertebrates and marine organisms. Field studies should also be reviewed and their results should be combined with the laboratory data in order to identify the safe concentrations of heavy metals in water. Merits and limitations of various statistical procedures used to determine these concentrations should be defined and discussed with reference to the application of standards to control the pollution.

The aquatic system reflects the perturbations in the environment. So, fish and invertebrates can often be used to indicate the health of aquatic system because chemicals can accumulate in the invertebrates from water and sediment, and in fish from water, sediment and food chain. Monitoring of these effects is extremely important to regulate and remediate pollution. To test the *'toxicity'*, they can apply *'biomarkers'* to detect low-level pollution in aquatic systems. There has to be developed the right biomarkers, and then they can be applied in the fish to detect pollution in contaminated groundwater with an aim to develop measures of toxic hazard and risk (Lenntech, 2013).

The *'histopathological changes'* are recognized and commonly used diagnostic tools in the study of *'aquatic toxicology'*. Oxygen consumption is widely considered to be a critical factor for evaluating the physiological response and a useful variable for an early warning for monitoring the aquatic organisms. The aquatic organisms maintain their oxygen consumption at a constant level along a gradient of environmental oxygen concentrations, until a *'critical oxygen concentration'* is reached and below which oxygen consumption begins to fall. Under stress conditions, this critical oxygen concentration is likely to increase, reflecting the capacity of aquatic organisms for coping with environmental perturbations (Yang et al., 2010).

Measuring heavy metals in aquatic organisms may be a bioindicator of their impact on organism and ecosystem health, but a true evaluation of the damage inflicted by heavy metals should come from comprehensive biomarker studies. Biomarkers are more telling than bioindicators as measurements of heavy metal contamination because they deal with chemical and physiological changes on the organism level and assess contamination based on a direct measure of change in the organism. Research over time has focused on various species and various biomarkers to determine the amount of heavy metal toxicity in aquatic environments. Sea anemones, sea urchins, grass shrimp and red mullet (benthic fish) have been used by various investigators for evaluation of such heavy metals. The biomarkers in mussels, such as glutathione (GSH) and *'metallothionein'* are often used to evaluate heavy metal contamination. Metallothionein is a low molecular weight, cysteine-rich protein that binds 7 moles of cadmium or zinc to every one mole of metallothionein in the aquatic organisms. It makes metallothionein a valued *in situ* biomarker of heavy metal pollution in the aquatic environments. The primary purpose of metallothionein in cells is to regulate copper and zinc homeostasis and to detoxify the cell of cadmium and mercury. Overexposure to heavy metal contaminants can lead to overproduction of metallothionein and consequently systemic damage to the organism. Although many species produce metallothionein and can be tested for metal toxicity via metallothionein measurements, the mussels showed higher rates of accumulation for metals than other species because of their filter feeding and sessile life histories. This has been shown to be especially true for cadmium (Wikipedia, 2013c).

In the water polluted with toxicants (e.g., heavy metals), growth of fish may be inhibited. The growth inhibition is one of the most distinct symptoms of toxic action of heavy metals on fish larvae. Therefore, fish body length and mass are the indicators of environmental conditions. The impact of toxic metals on the integrity and functioning of DNA has been investigated in several organisms. Many biomarkers have been utilized as tools for detection of exposure to genotoxic pollutants. Such biomarkers include the presence of DNA adducts, chromosomal aberrations, DNA strand breaks and micronuclei measurements. In fish, erythrocytes (RBCs) are mainly used as sentinel markers of exposure to genotoxic compounds (Khayatzadeh and Abbasi, 2010).

5

GENERAL TOXICITY OF HEAVY METALS

MOST TOXIC HEAVY METALS

Previously, the elemental impurity list of USP<231> included arsenic, lead, cadmium, mercury, copper, antimony, bismuth, molybdenum, silver and tin due to reactivity of these metals with sulphide ion utilized in the procedure. The metals were reported as heavy metals due to the procedural inability to show them discretely. In addition, arsenic, bismuth and molybdenum were not necessarily detected by USP<231> due to common occurrences of these elements in the forms inert to the mechanism in the procedure. Since many instrumental procedures have been developed over the life of USP<231> which give significant improvements in selectivity and sensitivity, the proposal of USP will require individual quantification of arsenic, lead, cadmium and mercury, which are *'most toxic'* to humans, animals and environment. If the presence of additional metals is suspected (e.g., if used in manufacturing process as catalysts or if detected during previous testing), then those additional metals would be added to the list. Each element screened will have individually distinct impurity limits, based on unique toxicity data (Pandey, 2013a).

In cooperation with the US *'Environmental Protection Agency'* (EPA), the *'Agency for Toxic Substances and Disease Registry'* (ATSDR, a part of the US Department of Health and Human Services) in Atlanta, Georgia reported that in a *'Priority List for 2011'* called the *'Top 20 Hazardous Substances'*, arsenic, lead, mercury and cadmium are at the 1st, 2nd, 3rd and 7th place, respectively in the list. Therefore, these 4 chemical elements are considered the *'most toxic heavy metals'* to humans, animals and aquatic lives (including fish), as well as to the environment (Lenntech, 2013).

NATURE OF TOXIC METALS

All heavy metals are not always toxic, and some of them are essential, e.g., iron. So,

the *'toxic metals'* may also include the *'trace elements'* when considered in abnormally high, toxic doses. The toxic metals sometimes imitate the action of an essential element in the body, interfering with the metabolic process to cause illness. Toxic metals can bioaccumulate in the body and food chain. Thus, a common characteristic of toxic metals is the chronic nature of their toxicity. Some metals are toxic when they form poisonous soluble compounds. Certain metals have no biological role, i.e., they are not essential minerals, or they are toxic when present in certain forms. However, any measurable amount of lead may have negative health effects (Pandey, 2013a). A *'poison'* is any substance that when absorbed into the body will cause adverse or deleterious effects. Many elements and their resulting compounds are known *'toxins'*, some of the most common and dangerous of which being arsenic, lead, mercury, copper (all metals) and selenium (non-metal) (Davis, 2010).

'Toxicity' is a function of solubility. *'Metal toxicity'* is the toxic effect of certain metals in certain forms and doses on the life. Both insoluble compounds as well as the metallic elements often exhibit negligible toxicity. However, the toxicity of any metal depends on its ligands. In some cases, the organometallic forms (e.g., methyl mercury and tetraethyl lead) can be extremely toxic. In other cases, the organometallic derivatives are less toxic, e.g., cobaltocenium cation. Decontamination for toxic metals is different from organic toxins because toxic metals are elements, they cannot be destroyed. The toxic metals may be made insoluble or collected, possibly by the aid of chelating agents. Alternatively, they can be diluted into a sufficiently large reservoir (e.g., sea) because immediate toxicity is function of concentration rather than amount. However, bioaccumulation has the potential to reverse this (Pandey, 2013a).

TOXIC MECHANISM OF HEAVY METALS

The exact mechanism of toxicity for most *'poisons or toxins'* is unknown; however, many poisons exert their toxic effects by distressing the enzyme systems of animal. Many toxins bind to specific enzymes and proteins necessary for cellular function and thus compete with other substances essential for maintenance and continued function of cells. Thus, the poisons can also have the effect of inducing mineral deficiencies. In

addition, many toxic substances appear to assist in the formation of paramagnetic anion, superoxide (O_2^-), which itself is toxic and seems widely responsible for spontaneous cell death (Davis, 2010).

Heavy metals can enter the body through inhalation, intestinal absorption and even be absorbed through the skin, depending upon their chemical form. The elemental forms of heavy metals are not well absorbed but the organometallic forms are lipophillic, and can readily pass through the membranes, and even cross blood-brain barrier (BBB, neurological defense system). Once absorbed into the body, the heavy metals have a wide distribution in various organs, glands and central nervous system (CNS). Some metals are *'bone seekers'*, and ultimately settle into teeth and skeletal system. The heavy metals then can effectively poison enzyme systems, increase *'free radical'* production and displace or compete with essential elements that make up metallo-enzyme complexes and compete with the absorption of nutritional minerals (Pandey, 2013a).

All metals are toxic to cells. They compete with *'nutritional minerals'* in body, thus rendering them unavailable to essential processes to maintain the health. For instance, in a hair analysis sample, aluminium (Al) displaces calcium and makes it unavailable for building bone, teeth and health of muscle function (including heart muscles), leading to weakening of these structures. Low calcium levels cause osteoporosis, dental caries, periodontal disease, muscle cramping, colic and heart disease. Other mineral disturbance is the lowering of zinc, phosphorous, manganese, iron and magnesium (Mg). Aluminium has a double whammy as it increases zinc and copper in the body to secondary toxic levels, which increase with every generation, even when the exposure level is same. It is because the growing foetus is far more vulnerable than the adult and most toxins pass through the placenta in-utero (Dodd, 2013; Eck and Wilson, 1989).

The study showed that the highest concentration of heavy metals is in liver and kidney of different fish species. The contaminated sediments can threaten creatures in the benthic environment, exposing worms, crustaceans and insects to hazardous concentrations of toxic metals. Some kinds of toxic sediments via killing the benthic organisms reduce the food availability for larger animals such as fish. Some contaminants in the sediment are taken up by benthic organisms through bioaccumulation. When larger

animals feed on these contaminated organisms, the toxins are taken into their bodies, moving up the food chain with increasing concentrations in a process known as *'biomagnification'*. The heavy metals are diluted and affected by various surface water components (carbonate, sulphate, organic compounds, amino acids, etc.) which formed insoluble salts or complexes. These salts and complexes are predicted to be not harmful to aquatic organisms. Part of them sink and are accumulated in bottom sediments. However, when water pH has declined (during acidic rains or other acidic episodes) heavy metals can be mobilized and released into the water column and become toxic to aquatic biota. In addition, the low concentrations of heavy metals can cause a chronic stress which may not kill individual fish, but lead to a lower body weight and smaller size and thus reduce their ability to compete for food and habitat. The aquatic organisms, e.g., fish, accumulate pollutants directly from contaminated water and indirectly via the food chain. Use of chemical fertilizers containing trace of heavy metals causes contamination of fish with these metals (Khayatzadeh and Abbasi, 2010).

COMMON TOXIC EFFECTS OF HEAVY METALS

Heavy metals contaminate the food, water and health supplements for animals and humans. They produce pathologies relative to quantity and period of time consumed. The main source of consumption is the pollution of air, water and soils by agribusiness, industry and public (Pandey, 2013a). While many heavy metals have toxic effects, others do not exhibit significant toxic properties. In fact, many of these elements including zinc, iron, copper, chromium and cobalt (Co) are necessary for metabolic function for variety of organisms. Although some heavy metals are *'essential micronutrients'* for animals, plants and many organisms, depending on the route and dose, all heavy metals show toxic effects on living organisms via metabolic interference and mutagenesis. The body health impacts range from reduction in fitness, to reproductive interference to carcinoma, with many exposures being lethal (Heavy Metals, 2013; Pandey, 2013a).

Toxicity of some heavy metals (e.g., lead and mercury) and deficiency of some essential trace elements (e.g., chromium) have been detected in some wild animal species with the help of inductively coupled plasma spectroscopy of hair and bone, cellular and

surface ultrastructural features of skin and hair, and behavioural studies on symptoms related to toxicity and deficiency of some elements. The values of elemental content indicating their toxicity or deficiency were found statistically significant. Electron microscopic studies on cellular and ultrastructural features of the skin and hair revealed specific toxic and deficiency effects of some elements. Behavioural studies showed many symptoms related to certain elemental disturbances such as loss of appetite, constipation, salivation, photophobia, tendency to wander in a circle, etc. The possible source of toxicity and deficiency of element were examined by analyzing soil and water samples from the home range of animals and also by studying the behaviour pattern in relation to mobility, migration and sequence of movements. Animals have very sensitive endocrine systems that are often even more intolerant to toxins than humans. The environmental factors are a major cause of such toxins (Pandey, 2013a).

'General toxicity' of some important heavy metals is described below. These *'toxic effects'* are more or less similar in all species of animals, including fish and humans.

Arsenic:

Arsenic is a known carcinogen which affects skin, liver, and digestive, nervous and respiratory systems. Accumulation of toxic levels of arsenic can result in paralysis, coma, cardiovascular collapse and death. *'Arsenic poisoning'* causes drooling, vomiting, bloody diarrhoea with mucus, bloody urine, muscle cramps, weakness, hair loss, skin rashes, gastrointestinal pain, convulsion, trembling and staggering. Exposure to high levels of inorganic arsenic (more than 100 ppm) with food or water can be fatal to human (Pandey, 2013a). This toxicity is of two kinds: organic and inorganic. Acute large dose of arsenic causes gastrointestinal damage with profuse watery diarrhoea, bleeding and death. Chronic, smaller dose of arsenic causes a non-specific wasting disease (arsenic is quintessential choice of poisoners because it mimics so many chronic diseases, which cannot be diagnosed except with hair analysis) (Dodd, 2013; Eck and Wilson, 1989).

Lead:

In humans, exposure to lead may produce a wide range of biological effects

depending on the level and duration of exposure. Various effects occur over a broad range of doses, with the developing foetus and infant being more sensitive than the adult. Although most people receive bulk of their lead intake from food, but in the specific population, other sources may be more important like water in areas with lead piping and plumb solvent water, air near point of source emissions, soil, dust and paint flakes in old houses or contaminated land. The lead in air contributes to lead levels in the food through deposition of dust and rain containing metal, on crops and soil. Eight broad categories of the use of lead are: batteries; petrol additives; rolled and extruded products; alloys; pigments and compounds; cable sheathing; shot; and ammunition. For infants up to 4 or 5 months of age, air, milk formulae and water are the significant sources of lead. The lead is among the most recycled non-ferrous metals, and its secondary production has, therefore, grown steadily in spite of declining lead prices. Its physical and chemical properties are applied in manufacturing, construction and chemical industries. It is easily shaped, and is malleable and ductile. High levels of lead exposure may result in toxic biochemical effects in humans which in turn cause problems in the synthesis of haemoglobin (Hb), effects on kidneys, gastrointestinal tract (GIT), joints and reproductive system, and acute or chronic damage to nervous system. At intermediate concentrations, however, there is persuasive evidence that lead can have small, subtle, subclinical effects, particularly on neuropsychological developments in children. There may be a loss of up to 2 *'intelligent quotient'* (IQ) points for a rise in the blood lead levels of 10 to 20 µg/dl in young children. Henceforth, the main symptoms of *'lead poisoning'* include poor bone growth, learning disability, anxiety, high blood pressure, gastrointestinal problem, muscle and joint pain, tremor, depressed immune function, decreased cognitive functioning, ringing in ear, anaemia, anorexia, convulsion, malaise, pallor, fatigue, weakness and weight loss. Other effects may be neurological (even at low level exposure in children), cardiovascular, renal, gastrointestinal, haematological and reproductive effects. Children 6 years old and below are most at risk (Pandey, 2013a).

Lead is one of the most danger elements to animal health. The lead-poisoned animal can be a risk to public health, since there is an accumulation of this mineral in meat and milk (Reis et al., 2010). *'Animal lead poisoning'* (also called as *'avian plumbism'* or

'avian saturnism' in birds) is caused by increased levels of the lead in the animal's body. This may be *'acute'* (from intense exposure of short duration) or *'chronic'* (from repeat low-level exposure over a prolonged period). The acute poisoning can quickly result in death (Pandey, 2013a). Dogs, cats and cattle, particularly young ones, are most likely to be affected by lead poisoning (Pandey, 2013a; The Merck Veterinary Manual, 2013).

Mercury:

Mercury is one of the most studied toxic heavy metals, and the lethal effects of both acute and chronic low-level exposures are well recorded. Main pathway for mercury to humans is through food chain and not by inhalation. The mercury at high levels may damage brain, kidney and developing foetus. To be safe from mercury toxicity, we must avoid the eating of too much fish, and pregnant women should avoid it completely due to the problems that mercury causes to the growing foetus. Mercury exposure can harm to brain, heart, kidneys, lungs and immune system of the people of all ages. In babies and young children, the nervous system can be affected making the child less able to think and learn. Some studies have suggested that methyl mercury may cause cancer in humans, but the results are inconclusive (Pandey, 2013a).

The main symptoms of *'mercury poisoning'* include insomnia, numbness, tingling, tremors, inhibition of transporting sugar, loosing of teeth, vomiting, blood changes, swallowing difficulties, mental depression, headache, hallucinations, fever, loss of sense of pain, chills, nephritis, blue line on gums, muscular weakness, asthma, multiple sclerosis, depressed immune system, impaired vision and hearing, irrational behaviour, allergic conditions, memory problems, anorexia, fatigue, weight loss, irritability and gastrointestinal disorders, etc. The inorganic mercury poisoning is associated with tremors, gingivitis and/or minor psychological changes, together with spontaneous abortion and congenital malformation in humans. Monomethyl mercury causes damage to brain and CNS; while foetal and postnatal exposures have given rise to abortion, congenital malformation and development changes in young children. Because of differences in tissue distributions, poisoning of mercury differs depending on whether it has been caused by exposure to elemental mercury, inorganic mercury compounds (as

salts), or organic mercury compounds. Mercury in its various forms like environmental toxins is particularly harmful to foetuses in pregnancy, as well as to infants. Women who have been exposed to mercury in substantial excess of dietary selenium intakes during pregnancy are at risk of giving birth to children with serious birth defects. The mercury exposures in excess of dietary selenium intakes in young children can have severe neurological consequences, preventing nerve sheaths from forming properly. Mercury inhibits the formation of myelin. As per some evidences, mercury poisoning may predispose to *'young syndrome'*, i.e., men with bronchiectasis and low sperm count (Pandey, 2013a; Pandey et al., 2012).

Cadmium:

Cadmium derives its toxicological properties from its chemical similarity to zinc (an essential micronutrient for plants, animals and humans). It is bio-persistent and, once absorbed by an organism, remains resident for many years (over decades for humans), although it is eventually excreted. It is produced as an inevitable by-product of zinc (or occasionally lead) refining, since these metals occur naturally within the raw ore. But once collected, the cadmium is relatively easy to recycle. The most significant use of cadmium is in the nickel-cadmium batteries, as rechargeable or secondary power sources exhibiting high output, long life, low maintenance and high tolerance to physical and electrical stress. Other uses of cadmium are as pigments, stabilizers for PVC, in alloys and electronic compounds. In general/non-smoking population, major exposure pathway is through food, via addition of cadmium to agricultural soil from various sources (atmospheric deposition and fertilizer application) and uptake by food and fodder crops. Additional exposure arises through cadmium in ambient air and drinking water. Long-term exposure of cadmium is associated with renal dysfunction. Its high exposure can lead to obstructive lung disease and has been linked to lung cancer. Cadmium may also cause bone defects (osteomalacia and osteoporosis) (Lenntech, 2013; Pandey, 2013a).

In humans, acute cadmium exposure normally occurs in workplace, particularly in the manufacturing processes of batteries and colour pigments used in paint and plastics, as well as in the electroplating and galvanizing processes. The main symptoms of

'cadmium poisoning' include fatigue, headache, high blood pressure, irritability, enlargement (non-cancerous) of prostate gland, pulmonary irritation, disorders of liver, kidney and skin, loss of hair, loss of smell and appetite, learning disability, possible cancer of lung, prostate and kidney, weakness of bones, and pain in joints. Specifically, the symptoms of acute cadmium poisoning are nausea, vomiting, abdominal pain and breathing difficulty. However, the symptoms of chronic cadmium poisoning include cardiovascular disease, chronic obstructive lung disease, renal disease, fragile bones, learning disorders, migraine, anaemia, growth impairment, emphysema, alopecia, arthritis, osteoporosis, and loss of taste, smell and appetite (Pandey, 2013a).

Besides the lung, liver and kidney diseases, cadmium also produces the reproductive disorders. It displaces zinc, producing poor healing and premature aging. The predilection of cadmium for stomach with reduced hydrochloric acid (HCl) has been produced by stomach, thereby rendering abnormal digestion of proteins which can lead to *'leaky gut syndrome'* and allergies (Dodd, 2013; Eck and Wilson, 1989).

Copper:

Copper is an essential mineral element, being the part of various enzymes and proteins. However, it is extremely toxic when ingested in excess. *'Copper poisoning'* can cause heavy economic loss due to mortality of animals (Reis et al., 2010). Copper can induce anaemia, liver and kidney damage, and stomach and intestinal irritation. People with Wilson's disease are at greater risk for health effects from overexposure to copper (Lenntech, 2013; Pandey, 2013a).

Chronic copper toxicity does not normally occur in humans because of transport systems which regulate the absorption and excretion. Autosomal recessive mutations in copper transport proteins can disable these systems, leading to Wilson's disease with copper accumulation and liver cirrhosis in persons who have inherited two defective genes. Symptoms of acute copper poisoning by ingestion include vomiting, haematemesis (vomiting of blood), hypotension (low blood pressure), melena (black *'tarry'* faeces), jaundice (yellowish pigmentation of skin), gastrointestinal distress and coma. The persons with glucose-6-phosphate deficiency may be at increased risk of haematologic

effects of copper. Haemolytic anaemia resulting from the treatment of burns with copper compounds is infrequent. In chronic copper poisoning, damage to liver and kidney may occur. Excessive amounts of copper can also cause testicular cancer (Pandey, 2013a).

Iron:

Iron usually damages cells in the heart, liver and elsewhere, which can cause significant adverse effects, including coma, metabolic acidosis, shock, liver failure, coagulopathy, *'adult respiratory distress syndrome'*, long-term organ damage and even death. Overconsumption of iron, often the result of children eating large quantities of ferrous sulphate tablets intended for adult consumption, is one of the most common toxicological causes of death in children under the age of 6 years. Although some studies suggest that haeme (haemoglobin) from red meat has effects which may increase the risk of colorectal cancer, there is still some controversy (Pandey, 2013a). *'Acute iron toxicosis'* is common and potentially lethal in dogs, cats and many other animals. Iron exerts its most profound effects on cardiovascular system. Excessive iron can cause post-arteriolar dilatation, increased capillary permeability, reduced cardiac output and fatty necrosis of myocardium. Histopathological examination of iron-induced hepatic damage reveals cloudy and swollen hepatocytes, portal iron deposition, fatty metamorphosis and massive periportal necrosis (Albretsen, 2006).

Molybdenum:

'Molybdenum poisoning' is also known as *'molybdenosis'*. In humans, mild cases of molybdenosis may be clinically identifiable only by biochemical changes (e.g., increase in uric acid level due to role of molybdenum in enzyme xanthine oxidase). Excessive intake of molybdenum causes a physiological *'copper deficiency'*, and conversely, in cases of inadequate dietary intake of copper, molybdenum toxicity may occur at lower exposure levels. The workers chronically exposed to molybdenum inhalation showed a high incidence of weakness, fatigue, headache, irritability, lack of appetite, pain in epigastric, joints and muscular parts, weight loss, red and moist skin, tremor of hands, sweating, and dizziness. Direct pulmonary effects like pneumoconiosis of chronic

exposure to molybdenum have been found in workers exposed to molybdenum and molybdenum trioxide. Animal data show that molybdenum may have an inhibitory effect on oesophageal and mammary carcinogenesis. Molybdenum is placed in EPA Group D, not classifiable as to carcinogenicity in humans (Opresko, 1993; Pandey, 2013a).

The ruminants that consume high amounts of molybdenum develop symptoms like anaemia, stunted growth, achromotrichia (loss of hair pigment) and diarrhoea. These symptoms can be lessening after administration of more copper into the system, both in dietary form and by injection (Pandey, 2013a). Cattle are more susceptible than sheep and horse because bovine (cattle) digestive tract has a good ability to absorb molybdenum, while horses have less capacity to absorb molybdenum. Accumulation of molybdenum in pituitary causes dysfunction in production and releases their hormones, which consequently cause reproductive alterations with infertility (Reis et al., 2010).

Zinc:

Although zinc is an essential requirement for good health, excess zinc can be harmful. Zinc is a common ingredient of denatured creams which may contain between 17 and 38 mg of zinc/g. These products can cause disability and even death in humans. Zinc damages nerve receptors in the nose, which can cause *'anosmia'*. This loss of smell can be life-threatening because people with impaired smell cannot detect leaking gas or smoke and cannot tell if food has spoiled before they eat it. The topical antimicrobial zinc pyrithione is a potent *'heat shock'* response inducer which may impair genomic integrity with induction of poly-ADP-ribose-polymerase dependent energy crisis in cultured human keratinocytes and melanocytes. In children, zinc causes growth retardation, delayed sexual maturation, infection susceptibility and diarrhoea. Excess zinc can cause ataxia, lethargy and *'copper deficiency'*. There is also a condition known as *'zinc shakes*, *zinc chills or metal fume fever'*, which is induced by inhalation of freshly formed zinc oxide during the welding of galvanized materials. Zinc levels of 100 to 300 mg may interfere with the utilization of copper and iron, or adversely affect cholesterol. *'Zinc poisoning'* has also been linked to alterations of blood lipoprotein levels, with increased levels of LDL and decreased levels of HDL. This toxicity may take months to resolve;

and nausea, vomiting, diarrheoa, pain and cramps, etc. are seen (Pandey, 2013a).

Manganese:

Higher level of manganese in drinking water is associated with increased intellectual impairment and reduced *'intelligence quotients'* (IQ) in school-age children. Methylcyclopentadienyl manganese tricarbonyl (MMT) is a gasoline additive used to replace lead compounds for unleaded gasoline. Manganese functions as an antiknock agent by the action of carbonyl groups. Fuels containing manganese tend to form manganese carbides, which damage exhaust valves. Use of such fuels directly or in mixture with non-reformed distillates is universal in developed countries. There was found childhood developmental disorder due to 240 to 350 µg Mn/L of drinking water; however, the children who received water from a well containing 610 µg Mn/L showed increased hyperactive and oppositional behaviour (Pandey, 2013a).

Antimony:

Antimony is used as antimony trioxide (a flame retardant). It is present in batteries, pigments, ceramics and glass. High levels of antimony for short periods cause nausea, vomiting and diarrhoea. Its long-term exposure may be carcinogenic to human (Lenntech**,** 2013; Pandey, 2013a). Inhalation of antimony trioxide (and similar poorly soluble antimony-III) dust particles (antimony dust) is harmful and may cause cancer. But this effect is only seen in female rats after long-term exposure to high dust concentrations. The effect is due to inhalation of poorly soluble antimony particles, causing impaired lung clearance, lung overload, inflammation and ultimately tumour formation. Antimony chlorides are corrosive to skin. Effects of antimony are not comparable to arsenic; this might be caused by significant differences of uptake, metabolism and excretion between arsenic and antimony. Antimony and its compounds do not cause acute health effects, with exception of tartar emetic (antimony potassium tartrate), a prodrug which is intentionally used to treat leishmaniasis patients. Prolonged skin contact with antimony dust may cause dermatitis. However, skin rashes are not substance-specific, but are most probably due to physical blocking of sweat ducts (Pandey, 2013a).

The occupational exposure by antimony in humans can cause respiratory irritation, pneumoconiosis, antimony spots on skin and symptoms of gastrointestinal tract (GIT). Besides these, antimony trioxide is possibly carcinogenic to humans. Improvements in working conditions have remarkably decreased the incidence of *'antimony toxicity'* at the work place. As a therapy, antimony has been mostly used for treatment of leishmaniasis and schistosomiasis. The major toxic side-effects of antimonials as a result of therapy are cardiotoxicity (about 9% of patients) and pancreatitis, which is seen commonly in HIV and visceral leishmaniasis co-infections (Sundar and Chakravarty, 2010).

In *'antimony poisoning'*, the harmful effects upon body tissues are seen. This poisoning resembles the *'arsenic poisoning'*. In antimony poisoning, the symptoms like depression, dizziness, headache, vomiting and kidney or liver damage are noticed. Antimony trioxide is even believed to be carcinogenic, and the antimony poisoning has also been said to cause *'Adams-Stokes syndrome'*. The trivalent antimony compounds (e.g., antimony potassium tartrate) are more toxic than the pentavalent ones. Antimony potassium tartrate can cause severe pain and tissue necrosis, and so it is not given by intramuscular or subcutaneous injection. Trivalent antimony compounds are toxic when used topically. The adverse effects are similar for all trivalent antimony compounds, and include nausea, vomiting, weakness and myalgia, abdominal colic, diarrhoea, and skin rashes including pustular eruptions. Hypersensitivity reactions also may occur. The respiratory symptoms are cough, dyspnoea and chronic lung changes. Cardiotoxicity is the most important and may produce arrhythmias, myocardial depression and damage, *'Adams-Stokes syndrome or attacks'*, heart failure, and cardiac arrest. Liver damage and necrosis, and blood dyscrasias may occur. In kidney, toxic effects may be seen after chronic use of antimony. Continuous treatment with small doses of antimony show the symptoms of subacute poisoning similar to those of *'chronic arsenic poisoning'* due to accumulation of antimony in body, especially if trivalent compounds are used, because of their long biological half-lives. Reproductive disorders and chromosome damage are also seen; hence, antimony compounds are severely toxic to reproduction, and have mutagenic and oncogenic potential. The pentavalent antimony compounds (particularly organic compounds, together with non-metallic synthetic preparations like diamidines) have

replaced the antimony potassium tartrate for treatment of leishmaniasis (Winship, 1987).

Chromium:

Chromium is generally used in the metal alloys and pigments for paints, cement, paper, rubber and other materials. The low-level exposure of chromium can irritate skin and can cause ulceration. The long-term exposure of chromium may cause kidney and liver damage. It can also damage the circulatory and nerve tissues (Lenntech, 2013; Pandey, 2013a). Chromium is essential to life at low concentrations, but it is toxic to many systems at higher concentrations. In addition to the overt symptoms of acute chromium toxicity, delayed manifestations of chromium exposure become apparent by subsequent increases in the incidence of various human cancers (Cohen et al., 1993).

Chromium (III) has benign effect; however, chromium in larger amounts and in different forms, e.g., hexavalent chromium [Cr (VI) or Cr^{6+}], can be toxic and carcinogenic. Chromium (VI) is very toxic and mutagenic when inhaled, but it is not recognized as a carcinogen when in solution, although it may cause allergic contact dermatitis. The popular dietary supplement chromium (III) picolinate complex generates chromosome damage in hamster cells due to picolinate ligand. Due to oxidation reactions, chromium (VI) damages blood cells, leading to haemolysis, and subsequently liver and kidney failure. The carcinogenicity of chromate dust has been known for a long time. Chromium salts (e.g., chromates) also cause allergic reactions in some people. The chromates are often used to manufacture leather products, paints, cement, mortar and anti-corrosives, besides other things. Hence, contact with these products may cause allergic contact dermatitis and irritant dermatitis, resulting in ulceration of skin (also called as *'chrome ulcers'*). This condition is usually found in workers who have been exposed to strong chromate solutions in electroplating, tanning and chrome-producing manufacturers. In Russia, pentavalent chromium, i.e., chromium (V) was reported as one of the causes of premature dementia (Pandey, 2013a).

The symptoms of acute chromium (VI) poisoning include the shortness of breathing, coughing and wheezing; whereas, the symptoms of chronic chromium (VI) poisoning include the perforations and ulcerations of septum, bronchitis, decreased pulmonary

function, pneumonia and other respiratory effects. Inhalation of chromium (VI) results in lung cancer (or tumour) in both human beings as well as in animals (Pandey, 2013a). The hexavalent chromium compounds of low and high water solubility may induce respiratory cancers and some other types of cancers. Since the hexavalent chromate is iso-structural with phosphate and sulphate, it is readily taken up by the GIT, and penetrates in many tissues and organs throughout the body of humans and animals. Epidemiological studies reveal that the hexavalent chromium may cause increased risk of bone, prostate, stomach, genitalia, kidney and bladder cancers, including lymphomas, leukemia and Hodgkin's disease. In addition, the non-cancer health effects of hexavalent chromium on the respiratory system, GIT, immune system, liver and kidney have also been observed. Hence, it shows that the hexavalent chromate penetrates all the tissues and organs of the body. A high accumulation of chromium (III) in all tissues and organs strongly indicates the wide toxic potential of exposure to soluble hexavalent chromium in the drinking water and ambient environment (Costa, 1997).

Nickel:

Small amounts of nickel are needed by human body to produce red blood cells (RBCs), but in excessive amounts, it can become mildly toxic. Short-term exposure to nickel is not known to cause health problems, but its long-term exposure can cause decreased body weight, heart and liver damage, and skin irritation (Lenntech, 2013; Pandey, 2013a).

6

TOXIC EFFECT OF HEAVY METALS ON AQUATIC ENVIRONMENTS

TOXIC HEAVY METALS OF AQUATIC ENVIRONMENTS

'Water' is one of the most valuable natural resources. The quality of water is of vital concern for the mankind since it is directly link with human welfare. A major environmental concern due to dispersal of industrial and urban wastes generated by human activities is the contamination of soil and water. Controlled and uncontrolled disposal of waste, accidental and process spillage, mining and smelting of metalliferous ores, sewage sludge application to agricultural soils are responsible for transferring of contaminants into non-contaminated sites as dust or leachate and contribute towards contamination of our ecosystem. The inorganic and organic compounds causing contamination include heavy metals, combustible and putrescible substances, hazardous wastes, explosives and petroleum products, phenol and textile dyes. The major components of inorganic pollutants are heavy metals. They have some different problems than the organic pollutants. The metal pollution of sea is less visible and direct than other types of marine pollution but its effects on marine ecosystems and humans are very extensive. The presence of metals varies between fish species depending on age, developmental stage and other physiological factors (Khayatzadeh and Abbasi, 2010).

'Aquatic pollution by heavy metals' is ubiquitous. This is due to both the natural abundance of metals within earth's crust and human activities. Some of them are of great toxicological concern, and have a wide range of toxic effects in both humans and animals, including fish. Some heavy metals, e.g., zinc and iron are required in trace amounts for many biological functions. Other heavy metals like lead and mercury have had significant toxic effects. The toxicity of heavy metals is produced by different means, such as from the drinking-water contamination (lead pipes), high ambient air

concentrations near emission sources, or intake via food chain. The heavy metals are dangerous as they tend to bioaccumulate. *'Bioaccumulation'* means an increase in the concentration of a chemical in a biological organism over time, compared to the chemical concentration in the environment. Most of the zoos which were once located on the outskirts of the cities and towns are now surrounded by human activities like vehicular traffic and industries, which can cause pollution and adversely affect the health and wellbeing of the wild/aquatic animals (Gupta, 2013; Lenntech, 2013 Pandey, 2013a).

The heavy metals like lead, copper and zinc are normal constituents of marine and estuarine environments. When additional quantities are introduced from industrial wastes or sewage, they enter the bio-geochemical cycle and, as a result of being potentially toxic, may interfere with the ecology of a particular environment. In different marine organisms, the behaviour of toxic heavy metals is described in terms of their absorption, storage, excretion and regulation when different concentrations are available in the environment. At higher concentrations, the detrimental effects of toxic heavy metals become apparent. Copper and zinc are essential for normal plant growth, although elevated concentrations of both essential and non-essential metals can result in growth inhibition and toxicity symptoms. Plants possess a range of potential cellular mechanisms that may be involved in the detoxification of toxic heavy metals and thus tolerance to metal stress (Heavy Metals in Aquatic System, 2013).

Steady population growth and industrialization have caused an increase in the metal pollutants in sediments and water column. The major anthropogenic sources of heavy metals in the aquatic environments are industrial and urban waste, wastewater discharges and shipping activity (Wikipedia, 2013c). Indiscriminate discharge of industrial effluents, raw sewage wastes and other waste pollutants damage the environments and affect the survival and physiological activities of target aquatic organisms. The metals have a tendency to accumulate and undergo food chain magnification, so heavy metals affect all groups of organisms and ecosystem processes, including microbial activities. Bioaccumulation of trace elements in living organisms and biomagnification in them describe the processes and pathways of these pollutants from one trophic level to another, exhibiting higher bioaccumulation ability in aquatic organisms. Some of these organisms,

e.g., fishes are consumed by human beings. They could also cause catastrophic diseases like *'Minamata'* and *'Itai-Itai'*. So, lowering of toxic heavy metals in aquatic environment should be monitored by accepted and updated methods (Baby et al., 2010).

TOXICITY OF HEAVY METALS INVOLVED IN AQUATIC ENVIRONMENTS

Exposure and ingestion *'environmental heavy metals'* may cause neurological and reproductive disorders, and other health problems both in humans as well as in animals. Ecosystem contamination from lead, cadmium and copper pollution can damage marine organisms at cellular level and possibly affect the ecological balance (Wikipedia, 2013c). Soil microorganisms can degrade organic contaminants, while metals need immobilization or physical removal. Although many metals are essential, all metals are toxic at higher concentrations, because they cause oxidative stress by formation of *'free radicals'*. Another reason why metals may be toxic is that they can replace essential metals in pigments or enzymes disrupting their function. Thus, metals render the land unsuitable for plant growth and destroy the biodiversity (Khayatzadeh and Abbasi, 2010).

Heavy metals in water are particularly dangerous for fish juveniles and may considerably reduce the size of fish populations or even cause extinction of entire fish population in the polluted reservoirs. Several data indicate that the heavy metals reduce survival and growth of fish larvae. They also cause behavioural anomalies, such as impaired locomotors performance resulting in increased susceptibility to predators or structural damages, mainly vertebral deformities (Khayatzadeh and Abbasi, 2010).

Toxic effects generally associated with chronic exposure of environmental heavy metals are mutagenicity, carcinogenicity, teratogenicity, immunosuppression, poor body condition and impaired reproduction. Domestic and wild animals (including fish) are used to assess the quality of environment and are sentinels of great importance for toxicological risk assessment. Many studies have been carried out to assess the exposure of heavy metals to wild animals. Both lead and cadmium accumulate in liver and kidney, but lead also accumulates in bone. A moderate use of liver and kidney from lead-exposed animals appears to present little or no health hazard. Utilization of these organs from the cadmium-exposed animals, however, should be avoided (Pandey, 2013a).

Some of the important environmental heavy metals causing toxicity to the aquatic organisms, including fish are dealt herewith.

Mercury:

Distribution and sources- Mercury has been well known environmental pollutant for many decades. As early as 1950's, it was proved that emission of mercury to environment could have serious effects on human health. These early studies explained that fish and other wildlife from different ecosystems generally receive toxic mercury levels when directly affected by mercury-containing emission from human-related activities. Human health concerns arise when fish and wildlife from these ecosystems are consumed by humans. Studied showed that fish (mainly from nutrient-poor lakes and often in very remote areas) usually have high mercury levels. The fish sampling surveys have also shown the widespread mercury contamination in streams, wet-lands, reservoirs and lakes. There are many sources of mercury to the environment, both natural and human-related. The natural sources include volcanoes, natural mercury deposits and volatilization from the ocean. The primary human-related sources are coal combustion, chlorine alkali processing, waste incineration and metal processing. The human activities have about doubled or tripled the amount of mercury in the atmosphere, and the atmospheric burden is increasing by about 1.5% per year (Krabbenhoft and Rickert, 2013).

Mercury is a global pollutant with complex and unusual chemical and physical properties. The major natural source of mercury is the degassing of the earth's crust, emissions from volcanoes and evaporation from natural bodies of water. World-wide mining of this metal leads to indirect discharges into the atmosphere. The usage of mercury is widespread in industrial processes and in various products (e.g., batteries, lamps and thermometers). It is also widely used in dentistry as an amalgam for fillings and by the pharmaceutical industry. Concern over mercury in the environment arises from the extremely toxic forms in which the mercury can occur. Mercury is mostly present in the atmosphere in a relatively unreactive form as a gaseous element. The long atmospheric lifetime (of the order of one year) of its gaseous form means the emission, transport and deposition of mercury is a global issue. There are historic instances of large

scale disasters, e.g., the mercury poisoning of waterways in Japan. The mercury poisoning (by methyl mercury) and *'Minamata disease'* had substantial neurological effects similar to *'Hunter Russell syndrome'* (Lenntech, 2013; Pandey, 2013a).

Bioaccumulation and toxicological concerns- Like many environmental pollutants, mercury undergoes *'bioaccumulation'*, which is the process by which organisms (including humans) can take up pollutants more rapidly than their bodies can eliminate them, so the amount of mercury in their body accumulates over time. If for a period of time an organism does not ingest mercury, its body burden of mercury will be reduced. If, however, an organism continuously ingests mercury, its body burden may reach at toxic levels. The rate of increase or decline in body burden is specific to each organism. For humans, about half the body burden of mercury can be eliminated in 70 days if no mercury is ingested during that period. The *'biomagnification'* is the incremental increase in concentration of a pollutant at each level of a *'food chain'*. Even at very low input rates to aquatic ecosystems that are remote from point sources, the effect of biomagnification may put the toxic levels of mercury. This phenomenon occurs because the food source for organisms higher on the food chain is progressively more concentrated in mercury and other pollutants, thus magnifying bioaccumulation rates at the top of food chain. The effect of bioaccumulation is normally compounded the longer an organism lives, so that larger predatory *'game fish'* will likely have the highest mercury levels. This is because of the fact that mercury deposits in the fish muscle tissues. So, unlike organic pollutants (e.g., PCBs and dioxins) which concentrate in the skin and fat, mercury cannot be filleted or cooked out of consumable game fish (Krabbenhoft and Rickert, 2013).

Therefore, fish accumulate large amounts of mercury in their tissues, and represent a major dietary source of this element for humans. Fishes are the single largest and first sources of mercury contamination for humans. Biotransformation of mercury and methyl mercury formation constitutes a dangerous problem for human health (Khayatzadeh and Abbasi, 2010). Natural biological processes can produce methylated forms of mercury, which bioaccumulate over a million-fold and concentrate in living organisms, especially fish. These mercury forms (monomethyl mercury and dimethyl mercury) are highly toxic, causing neurotoxicological disorders (Lenntech, 2013; Pandey, 2013a).

Mercury cycle in aquatic ecosystems- The mercury cycling pathways in the aquatic environments (Fig. 43) are very complex. Different forms of mercury can be converted from one to the next; most important is the conversion to methyl mercury (CH_3Hg^+), the most toxic form. Ultimately, mercury ends up in the sediments, fish and wildlife, or evades back to the atmosphere by volatilization. With exception of isolated cases of known point sources, the ultimate source of mercury to most aquatic ecosystems (environments) is deposition from the atmosphere, primarily associated with rainfall. As shown in Figure 43, the atmospheric deposition contains 3 main forms of mercury, although the majority is as inorganic mercury (Hg_2^+, ionic mercury). Once in surface water, mercury enters a complex cycle in which one form can be converted to another. It can be brought to the sediments by particle settling and then later released by diffusion or resuspension. It may enter the food chain, or it can be released back to atmosphere by volatilization. The concentrations of *'dissolved organic carbon'* (DOC) and pH have a strong effect on the ultimate fate of mercury in an ecosystem. For the same species of fish taken from the same region, increasing the water acidity (decreasing pH) and/or DOC content normally results in higher body burdens in fish. Many scientists state that higher acidity and DOC levels increase the mobility of mercury in the environment, thus making it more likely to enter the *'food chain'*. Many details of aquatic mercury cycle are still unknown (Krabbenhoft and Rickert, 2013).

Entrance of mercury to food chain- The exact mechanism by which mercury enters the *'food chain'* is unknown, and can vary among ecosystems. However, it is stated that the bacteria that process sulphate ($SO_4^=$) in environment take up mercury in its inorganic form, and through metabolic processes convert it to methyl mercury. Conversion of inorganic mercury to methyl mercury is important for two causes: (a) methyl mercury is much more toxic than inorganic mercury; and (b) organisms need longer to eliminate methyl mercury. At this juncture, methyl mercury containing bacteria can be consumed by the next higher level in food chain, or the bacteria may release methyl mercury to water where it can quickly adsorb to plankton, which are also consumed by the next level in food chain (Krabbenhoft and Rickert, 2013). The process of mercury consumption via food chain by the fish and other aquatic animals is illustrated in Figure 44.

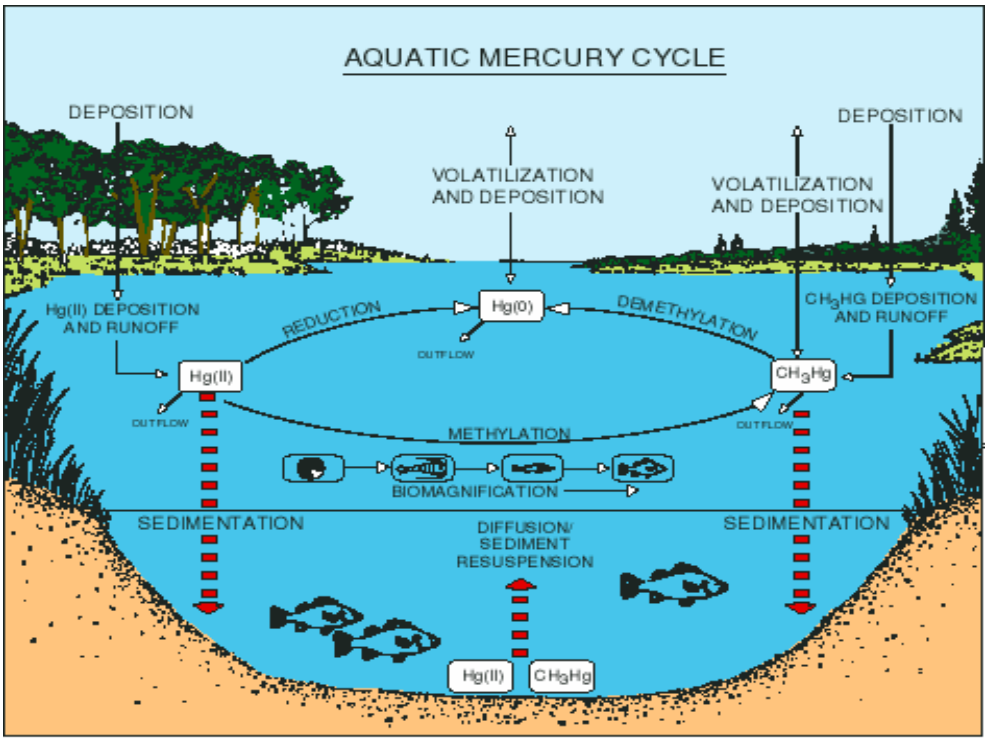

Fig. 43: Mercury Cycle in Aquatic Ecosystems

[Source of figure: Google website- Mercury Pollution, Lewis Publishers, CRC Press that is gratefully acknowledged]

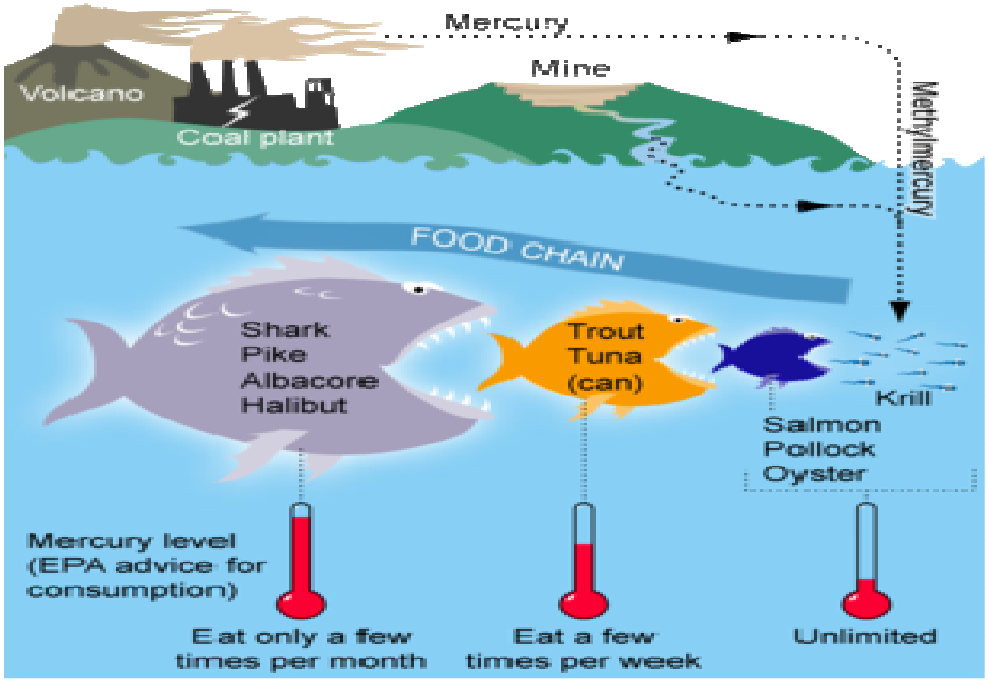

Fig. 44: Mercury Consumption via Food Chain by Fish and other Aquatic Animals

[Source of figure: Google website which is gratefully acknowledged]

Many evidences suggest that increased emissions of mercury to atmosphere, and its subsequent higher deposition to lakes, may cause higher mercury levels in fish. This mercury is strongly absorbed by organic materials (e.g., plankton or bacteria) floating in the water. These materials are consumed by the organisms higher in the food chain, or after dying, settle to the bottom of the lake and are incorporated into bottom sediments. The younger sediments deposited since industrialization have mercury concentrations which are about 3 to 5 times that of historical sediments. Thus, the fact that these sediments are primarily composed of dead microorganisms that were once the bottom of the food chain would suggest that modern levels of mercury in the food chain are elevated over pre-industrial times (Krabbenhoft and Rickert, 2013).

Arsenic:

Arsenic is released into environment by smelting process of copper, lead and zinc, as well as by manufacturing of chemicals and glasses. It is a common element which occurs in air, water, soil and all living tissues. Major sources of arsenic release to environment include coal fired power plants and arsenic-treated lumber. Arsenic can be found in many commonly used products, including fungicides, pesticides, herbicides, laundry products, secondhand cigarette smoke, paints and wood preservatives. Global industries like mining and smelting, chemical and glass manufacturing produce arsenic like byproduct. This in turn finds its way into our water supplies and food sources (Heavy Metals in Aquatic System, 2013; Pandey, 2013a). Through water supplies, arsenic may expose to shellfish, cod and haddock. It can easily contaminate water, and arsenic concentrated water is a serious threat in countries like Bangladesh (Davis, 2010).

Arsenic is a carcinogen which can cause foetal death and malformations in many species of mammals (Pandey, 2013a). Fish are the single largest sources of arsenic contamination, similar to mercury for humans (Khayatzadeh and Abbasi, 2010).

Lead:

Lead has worldwide distribution, and is accumulated in the environment by industrial pollution. The lead of air deposits in the food, crops and soil through deposition of dust

and rain containing lead. In environment, lead arises from both natural and anthropogenic sources. Its exposure can occur through drinking water, food, air, soil and dust from old paint containing lead (Lenntech, 2013; Pandey, 2013a). Total lead emissions have been decreasing in most industrialized countries and release to aquatic environment decreased due to improved treatment of wastewater. Open burning of waste products containing lead is probably an important source of local and regional lead emissions to the atmosphere as well as illegal dump sites and disposal in wet-lands and rivers. Use of lead, e.g., in manufacturing of plastics and paints in some developing countries is continued or increased (Heavy Metals in Aquatic System, 2013; Pandey, 2013a).

The health effects from lead exposure and impact on development may cause significant economic losses for society. In the environment, lead bioaccumulates in most organisms and is toxic to plants, animals and microorganisms. Lead is one of the most frequent and dangerous sources of toxicity to animals. It can be found in all parts of our environment. The young fish are more susceptible to lead poisoning than mature fish or eggs. The symptoms of lead toxicity in fish include spinal deformity and blackening of the caudal region, i.e., rear part of the fish (Pandey, 2013a).

Cadmium:

Cadmium is accumulated in the environment by industrial pollution, which is responsible for soil contamination and also contaminates pasture because some plants can accumulate cadmium, so it is ingested by animals grazing these plants. These contamination sources of cadmium are constantly introduced into the environment (e.g., water, air and soil) and can cause poisoning (Reis et al., 2010). Cadmium pollution is becoming more common due to its presence in drinking water, cigarette smoke, auto tires and processed meats (Dodd, 2013). Human activities have caused elevated cadmium concentration in environment. In agricultural soils, cadmium is mainly deposited through atmospheric deposition, phosphate fertilizers and sewage sludge. Besides, people with high intake of shellfish and organ meat from marine animals have higher cadmium levels. Cadmium is also toxic to plants, animals and microorganisms. Plants consume cadmium, which can result in an increase concentration in food products (Pandey, 2013a).

Hence, cadmium may also be found in reservoirs containing shellfish. Although cadmium is extremely rare in the earth's crust, pollution from industry processes has allowed this toxic metal access into the soils and water supplies (Davis, 2010).

Cadmium is present as an impurity in many products, including phosphate fertilizers, detergents and refined petroleum products. Its coatings provide good corrosion resistance, particularly in high stress environments like marine and aerospace applications, where high safety or reliability is needed; coating is preferentially corroded if damaged (Lenntech, 2013; Pandey, 2013a). **C**admium accumulates mainly in kidney and liver of vertebrates and in aquatic invertebrates and algae. Its acute toxic effects on fish, birds and other animals may be death or foetal malformation (Pandey, 2013a).

Copper:

Copper is a naturally occurring metal found in the earth**'**s crust. It is also generally present in surface waters, with cupric ion (Cu^{+2}) as the primary form in natural surface waters. In freshwater systems, naturally occurring concentrations of copper range from 0.2 µg/L to 30 µg/L. Copper may be released into environment through copper mining activities and agricultural activities (e.g., through its use as herbicide, fungicide, and/or algaecide), and manufacturing activities (e.g., manufacturing of leather and leather products, fabricated metal products, electrical equipment and automobile brake pads). Copper may also enter the environment through natural processes, e.g., volcanic eruptions, windblown dusts, decaying vegetation, and forest fires. Besides, copper is present in most municipal effluents due to corrosion of copper plumbing (Heavy Metals in Aquatic System, 2013; Wikipedia, 2013c). Copper and its alloys have been used for thousands of years. Copper is essential to all organisms as a trace dietary mineral, because it is a key constituent of respiratory enzyme complex *'cytochrome-c-oxidase'*. In mollusks and crustaceans, copper is a constituent of blood pigment *'haemocyanin'*, which is replaced by the iron-complexed haemoglobin in fish and other vertebrates. The rich sources of copper are beef and lamb liver, Brazil nuts, blackstrap molasses, cocoa, black pepper and oysters. Other good sources are nuts, sunflower seeds, green olives, avocados, wheat bran and lobster. Copper normally occurs in drinking water from copper pipes and

additives designed to normally occurs in drinking water from copper pipes and additives designed to control algal growth (Lenntech, 2013; Pandey, 2013a).

High levels of copper are toxic in aquatic environments, and may adversely affect the fish, invertebrates, plants and amphibians. The acute toxic effects of copper may include mortality of organisms; while the chronic toxicity copper can cause reduction in survival, reproduction and growth (Heavy Metals in Aquatic System, 2013; Wikipedia, 2013c).

Zinc:

Zinc plays important role in several enzymatic processes of body. It is *'ubiquitous'* in nature and exists in many forms. Zinc exposures normally occur from the dietary sources. The household sources of zinc are paint, batteries, automotive parts, zinc oxide creams, vitamin and mineral supplements, zipper pulls, board-game pieces, pet carrier screws and nuts, and coating on certain types of pipes and cookware (Pandey, 2013a).

Zinc is most toxic to microscopic organisms in the aquatic environments. It is also an essential element for aquatic and terrestrial biota, and its removal from the environment below certain levels can also be harmful due to its deficiency. Zinc may bind to particulate matter. Soluble species of zinc are readily available for biological reactions and, therefore, considered as most toxic. Zinc in water is a better predictor of fish tissue contamination than zinc in either sediment or invertebrates, i.e., food source. *'Free zinc ion'* is highly toxic to plants, invertebrates and even vertebrate fish. The *'free ion activity model'* shows that just micromolar amounts of free zinc ion kill some organisms. A study showed that 6 micromolar of free zinc ion killed 93% of all *Daphnia* in water (Heavy Metals in Aquatic System, 2013; Pandey, 2013a; Wikipedia, 2013c).

Manganese:

Manganese has a great environmental health concern. Most broad-spectrum plant fertilizers contain manganese; water-borne manganese; fuel (gasoline) additive methylcyclopentadienyl manganese tricarbonyl (MMT), which on combustion becomes partially converted into manganese phosphate and sulphate that go air-borne with the exhaust; fuels containing manganese carbides; racing fuel (e.g., cart and mini-bike)

manganese-containing drugs; manganese-contaminated drinking water; and manganese ethylene-bisdithiocarbamate (Maneb), a pesticide. Water-borne manganese has a greater bioavailability than the dietary manganese. The MMT contains 24.4 to 25.2% manganese. There is strong correlation between elevated atmospheric manganese concentrations and automobile traffic density (Pandey, 2013a; Wikipedia, 2013c).

Manganese is a naturally occurring substance present in the surface waters and biota. Aquatic organisms have shown toxic responses to manganese in surface waters. Acute and chronic toxicity tests for manganese were done on fish, invertebrates and freshwater algae. Manganese, an essential trace element for aquatic and terrestrial biota, is slightly to moderately toxic for aquatic organisms in excess amounts. It is present in almost all organisms, and often ameliorates the hazard posed by other metals. Nevertheless, manganese concentrations in environment may be well above the aquatic toxicity levels in effluents originating from base and precious metal mines, municipal sewage and sludge, and landfills. Manganese binds to particulate matter, and 90 to 95% of the total waterborne manganese residue is associated with particulate matter. However, soluble species of manganese are considered to be the most toxic as they are readily available for biological reactions (Heavy Metals in Aquatic System, 2013; Wikipedia, 2013c).

Antimony:

Antimony is found in nature mainly as sulphide stibnite (Sb_2S_3). The antimony compounds have been known since ancient times and were used for cosmetics. About 60% of antimony is consumed in flame retardants, and 20% is used in alloys for batteries, plain bearings and solders. Main sources of antimony are drinking water, batteries, plain bearings, flame retardants, microelectronics, solders, stabilizer and pigments (Pandey, 2013a). Diantimony trioxide may be dissolved in the atmosphere and the trivalent form of antimony is oxidized to its pentavalent form. Antimony is deposited from atmosphere is predominantly dissolved in rain, but also as particulate matter in wet and dry deposition. Diantimony trioxide is released to environment via emissions to air, waste water, surface water and soil from its manufacture, formulation, processing, use and disposal, but also via coal combustion and refuse incineration, non-ferrous metal production (e.g., copper)

and road traffic (Heavy Metals in Aquatic System, 2013; Wikipedia, 2013c).

Antimony is widely spread in the aquatic environment. The trivalent forms of antimony are more toxic than its other forms. Antimony potassium tartrate (tartar emetic) is more toxic to planktonic crustaceans than the fish and green algae, and planktonic crustaceans appears a better indicator of antimony pollution in the aquatic environment (Nam et al., 2009). The massive antimony does not affect human health and environments. However, inhalation of antimony trioxide (and similar poorly soluble antimony-III) dust particles (antimony dust) is harmful and suspected to cause cancer. Most antimony compounds do not bioaccumulate in the aquatic life (Lenntech, 2013; Pandey, 2013a). But antimony has been reported as a potential pollutant in aquatic environments, causing many toxic effects on the aquatic animals (Yang et al., 2010).

Chromium:

Chromium in the environment is mainly in two valence states: insoluble trivalent chromium (chromium-III); and soluble hexavalent chromium (chromium-VI). Chromium (III) is much less toxic than chromium (VI). Chromium exposure may occur from natural or industrial sources. Chromium occurs in trace amounts in foods and waters. It is used in metal alloys and pigments for paints, cement, paper, rubber and other matters (Lenntech, 2013; Pandey, 2013a). Chromium is widely used in several industrial processes, and as a contaminant of many environmental systems (Cohen et al., 1993).

Intense industrialization and other anthropogenic activities have led to the global occurrence of soluble chromium (VI), which is readily leached from soil to groundwater or surface water, in concentrations above permissible levels. All chromium (VI)-containing compounds were once thought to be man-made, with only chromium (III) naturally ubiquitous in air, water, soil, and biological materials. The naturally occurring chromium (VI) has been found in ground and surface waters at values exceeding the World Health Organization (WHO) limit for drinking water of 50 μg Cr(VI)/L. The combustion of fossil fuels and manufacturing processes of iron and steel industries releases chromium into the atmosphere in particulate form. Most of the chromium in air eventually settle and end up in waters or soils. When released to land, chromium

compounds bind to soil and are not likely to migrate to ground water. In water, however, these compounds are very persistent as sediments, with a high potential for accumulation of chromium in aquatic life. In its dissolved form, chromium is present as either the anionic trivalent $Cr(OH)_3$ or hexavalent CrO_4^{2-} (Velma et al., 2009).

The *'ecotoxicology'* of chromium (VI) is linked to its environmental persistence and the ability to induce various adverse effects in biologic systems, including fish. In aquatic ecosystems, chromium (VI) exposure poses a significant threat to aquatic life. Chromium (VI) is a toxic industrial pollutant and classified carcinogen possessing mutagenic and teratogenic properties. The research indicates that chromium exposure can induce a variety of adverse effects in fish at physiologic, histologic, biochemical, enzymatic and genetic levels. Certain fish species, however, appear to show more sensitivity to chromium toxicity than others. Thus, chromium-induced toxicological pathology in fish is influenced by such factors as species, age, environmental conditions, exposure-time and exposure-concentration. The exact causes of fish death are multiple and depend mainly on time-concentration combinations (Velma et al., 2009). Chromium often accumulates in the aquatic life, adding to the danger of eating fish that may have been exposed to the high levels of chromium (Lenntech, 2013; Pandey, 2013a).

Nickel:

Nickel is a ubiquitous trace metal and occurs in soil, water, air and biosphere. Its prevalent ionic form is nickel (II). Most nickel is used for production of stainless steel and other nickel alloys with high corrosion and temperature resistance. Nickel alloys and nickel platings are used in vehicles, processing machinery, armaments, tools, electrical equipment, household appliances and coinage. Nickel compounds are also used as catalysts, pigments and in batteries. Primary sources of nickel emissions into ambient air are combustion of coal and oil for heat or power generation, incineration of waste and sewage sludge, nickel mining and primary production, steel manufacture, electroplating, and miscellaneous sources like cement manufacturing. Nickel from different sources ultimately reaches to waste waters. The residues from waste-water treatment are disposed of by deep well injection, ocean dumping, land treatment and incineration. The entry of

nickel into aquatic environment is by removal from the atmosphere, surface run-off, discharge of industrial and municipal waste, and by natural erosion of soils and rocks. In rivers, nickel is mainly transported in the form of a precipitated coating on particles and in association with organic matters. Nickel occurs in aquatic systems as soluble salts adsorbed on clay particles or organic matters (detritus, algae, bacteria, etc.), or associated with organic particles (e.g., humic and fulvic acids, and proteins). Absorption processes may be reversed leading to release of nickel from the sediment (Heavy Metals in Aquatic System, 2013; Wikipedia, 2013c).

Nickel can accumulate in the aquatic life, but its presence is not magnified along the food chains (Lenntech, 2013; Pandey, 2013a). *'Nickel toxicity'* in aquatic invertebrates varies considerably according to species and abiotic factors. Nickel is known to accumulate in the sediments. The chronic exposure of nickel has been found to cause significant toxic effects on spawning in mysid shrimp (*Mysidopsis bahia*), as well as in several aquatic organisms including fish (Heavy Metals in Aquatic System, 2013; Wikipedia, 2013c).

7

TOXICITY OF CERTAIN HEAVY METALS IN FISH

HEAVY METALS CAUSING TOXICITY TO FISH

Heavy metals concerned with aquatic environments mainly include lead, arsenic, mercury, cadmium, chromium, copper, zinc, manganese, antimony, nickel and silver, etc. Most of the heavy metals are poisonous to fish but some are less toxic, e.g., bismuth. All heavy metals, in spite some are essential micronutrients, have toxic effects on fish via metabolic interference and mutagenesis. Whilst, both insoluble compounds and metallic forms of heavy metals usually have no toxic effect. Organometallic forms, e.g., methyl mercury and tetraethyl lead, can be highly toxic; but organometallic derivatives are less toxic, e.g., cobaltocenium cation. Bioaccumulation of toxic metals can occur in body and food chain. So, toxic metals normally exhibit chronic toxicity, e.g., radioactive heavy metals like radium can imitate calcium to be incorporated into bone, but the similar health hazards can also be due to lead or mercury. However, barium (Ba) and aluminium are exceptions as they can be quickly excreted by kidney. Industrialization has spoiled the environment by putting more and more concentrations of many metals. Mercury and lead heavy metals cause severe toxicity to fish, as there are some historic cases like mercury poisoning of waterways in Japan (Madhuri et al., 2012b; Pandey et al., 2013).

Some heavy metals, such as iron, copper, zinc and manganese are essential for biological systems like enzymatic activities; whereas, other heavy metals like lead, mercury and cadmium, have no known important role in living organs and are toxic even in trace amounts. The essential metals are taken up from water, food or sediment by fishes for their normal metabolism; however, these metals can also have adverse and toxic effects at high concentration (Dobaradaran et al., 2010).

HEAVY METALS POLLUTION TO FISH

Pollution has become a serious threat, and has brought hazards to growing population

and environment. The speedy urbanization and industrialization has led to increased disposal of pollutants like heavy metals, radio nuclides, and various types of organic and inorganic substances into the environment. The heavy metals constitute major pollutants in environment. They are important pollutants for fish, because they are not eliminated from aquatic systems by natural methods like organic pollutants, and are enriched in mineral organic substances. The metal pollutants are mixed in the aquatic system through smelting process, effluents, sewage and leaching of garbage which cause severe harm to aquatic system. Tannery industry has added pollutants to aquatic environment. The tannery waste waters continue to cause hazardous effects on the aquatic organisms as they also have endocrine disruption effects. A large number of chemicals are being used by the tanners during process, and thus discharge the toxic materials into the waters. Due to this, agricultural lands are also degraded. Uncontrolled release of tannery effluents has increased the health risks to different organisms (Praveena et al., 2013).

Heavy metals pollution of aquatic environment has become a great concern. Because of toxicity and accumulative behaviour of heavy metals, they can make different changes in aquatic environment, such as species diversity. With increasing heavy metals in the environment, these elements enter the biogeochemical cycle. They can enter into the water via drainage, atmosphere, soil erosion and all human activities by different ways. They may enter from contaminated water into the fish body by different routes and accumulate in different organs of fish (Dobaradaran et al., 2010).

Heavy metal contamination may have devastating effects on the ecological balance of the recipient environment and a diversity of aquatic organisms. Because of the pollutants, a huge mortality occurs in different fish species. The presence of metals in fish species depends on the age and development of fish, and other physiological factors. The heavy metals released from domestic, industrial and other man-made activities can highly contaminate the aquatic systems. Such contaminations can seriously affect the ecological balance and diversity of aquatic species. For evaluation of health of aquatic systems, fish are widely used since pollutants present in food chain cause ill-effects and death of aquatic animals. Thus, the problem of metal pollution is one of the major health problems in the persons who eat sea foods (Madhuri et al., 2012a).

Fish diversity of any regime has great significance in assessment of that zone reference to environment and pollution, as well as it contributes to the necessary information for fisheries. Many fishes may be the bioindicators of environmental pollutants also (Pandey, 2013b). However, in the conservation of fish diversity, it is essential to protect the fish from the environmental toxic heavy metals, as fishes are most often contaminated by these pollutants. The toxic metals like mercury accumulate in the aquatic system, and can be toxic to humans or animals when they eat fish. The toxic materials must be handled with precautions and be disposed of properly. More often, heavy metals are mixed in fertilizers; however, the toxic levels of such metals are absorbed by some plants, ultimately consumed by humans resulting into detrimental effects on children (Madhuri et al., 2012b).

TOXIC EFFECTS OF SOME HEAVY METALS IN FISH

The toxic effects of heavy metals generally include the reduction in fitness, interference in reproduction leading to carcinoma and finally death. In fish, *'mercury toxicity'* (by methyl mercury) and *'Minamata disease'* exhibited significant neurotoxicity similar to *'Hunter Russell syndrome'*. Besides direct toxicity of heavy metals, the significant reduction in foetal growth and chronic effects are also observed. Much of the basic research relies on studies in different animal species. Many heavy metals (including essential ones) are poisonous above their threshold levels. The heavy metals usually enter the body through respiration, ingestion and skin (Madhuri et al., 2012b). Excessive amounts of heavy metals are detrimental as they destabilize ecosystems because of their bioaccumulation in the organisms, and elicit toxic effects on the biota and even death of most living organisms (Gupta, 2013).

Arsenic and inorganic arsenic compounds, cadmium compounds, nickel compounds, crystalline forms of silica, and beryllium and its compounds are chemical carcinogens, causing cancer in fish. The polluted marine organisms used as sea foods have caused health hazards, including neurological and reproductive disorders in both humans and animals. Water contaminated with mercury can cause abnormal behaviour, slower growth and development, decreased reproduction, and death in fishes. The persistent organic

pollutants can cause illness, deformities and deaths in fishes (Madhuri et al., 2012b). *'Arsenic toxicity'* is one of the most important toxicological hazards to animals which can occur due to arsenic trioxide, arsenic pentoxide, sodium and potassium arsenate, sodium and potassium arsenite, and lead or calcium arsenate. Drinking water containing more than 0.25% arsenic is potentially toxic. Arsenic seems to prefer tissues rich in oxidative enzymes such as liver, kidney and intestine. Lethal doses of sodium arsenite in most species of animals are 1 to 25 mg per kg (Uppal, 2000). In a study on the spotted snakehead fish (*Channa punctatus*, Bloch), it was seen that when high concentration (2 mM) of sodium arsenite (NaAsO) affected these fishes, they died within 2.5 hr. The chromosomal DNA of liver cells were fragmented which indicated that sodium arsenite might have caused death of those cells through apoptosis. The fishes were found particularly susceptible to arsenic toxicity when they were continually exposed to it through gills and arsenic-contaminated food. Sensitive aquatic species were damaged by 19 to 48 µg As/L water, 120 mg As/kg diet, or (in case of freshwater fish) tissue residues >1.3 mg/kg fresh weight (Madhuri et al., 2012a).

Stominska and Jezierska (2000) reported that exposure of lead and copper to common carp (*Cyprinus carpio*) larvae resulted in slowed down development and growth rate, and reduced survival; copper inhibited the skeletal ossification, while lead caused the scoliosis. Emami Khansari et al. (2005) explained that canned tuna fish (as a predator) is able to concentrate the large amounts of heavy metals, e.g., arsenic, mercury, lead, cadmium. Hayat et al. (2007) on three species of main carps (*Catla catla, Labeo rohita* and *Cirrhina mrigala*) observed that they had negative growth with weight in exposing to sublethal concentrations of manganese for 30 days. In fish, the toxic effects of heavy metals can influence the physiological functions, individual growth, reproduction and mortality. High concentration of manganese detected in the gills of these fish species showed that the main route of manganese uptake was through gills because little absorption of this metal occurred through gut via food. Further, long-term exposure (20 days or more) to waterborne cadmium at sublethal concentrations showed decreased growth in juvenile and adult rainbow trout, *Oncorhynchus mykiss*.

Vinodhini and Narayanan (2009) reported that heavy metals significant increased the

red blood cells (RBCs), and concentrations of blood glucose and total cholesterol in common carp (*C. carpio*). The levels of serum iron and copper were increased. The activity of vitamin C was decreased during chronic exposure to toxic heavy metals, which indicates the presence of reactive oxygen species (ROS) induced per oxidation. Hence, the presence of toxic heavy metals in the aquatic environment has strong influence on the haematological parameters in common carp freshwater fish. Daei et al. (2009) observed that cadmium significantly ($P<0.05$) replaced the ferritin (Fe) over the time in fish (*Chalcalburnus chalcoides*) blood but the lead could not do so. The results indicated that by increasing lead density in fish, this metal was absorbed by other tissues. In an experimental study (Nam et al., 2009), the effects of antimony potassium tartrate (a trivalent inorganic form of antimony) on Japanese medaka (*Oryzias latipes*), planktonic crustacea (*Moina macrocopa* and *Simocephalus mixtus*) and green algae (*Pseudokirchneriella subcapitata*) were evaluated. The larval survival and embryonic development were measured for fish assay. Antimony potassium tartrate was found to be less toxic to larval medaka (24-hr LC_{50}, 261 mg $L^{(-1)}$; 48-hr LC_{50}, 238 mg $L^{(-1)}$). *S. mixtus* was killed by very low concentrations of antimony potassium tartrate (24-hr LC_{50}, 4.92 mg $L^{(-1)}$). The antimony potassium tartrate was also found to be toxic to *M. macrocopa* (24-hr LC_{50}, 12.83 mg $L^{(-1)}$). The toxicities of antimony potassium tartrate to *S. mixtus* and *M. macrocopa* were about 50 and 20 times more to *O. latipes* larvae, respectively in terms of 24-hr LC_{50} value. The growth inhibition of *Ps. subcapitata* was seen in the presence of antimony potassium tartrate (72-hr EC_{50}, 206 mg $L^{(-1)}$).

Dobaradaran et al. (2010) determined the levels of lead, cadmium, copper and nickel in muscle and skin of two important consumed fishes (Indo-Pacific king mackerel and Tigertooth croaker). The results showed that the highest and lowest contents of heavy metals in muscle and skin of both fish samples were related to concentrations of cadmium and copper, respectively. The heavy metal contents in both skin and muscle samples of Tigertooth croaker were found to decrease in sequence as Cu>Pb>Ni>Cd. Montaser et al. (2010) reported histopathological lesions in fishes exposed to lead, cadmium, copper and zinc. Liver revealed hepatocytes vacuolation, cellular swelling, nuclear degeneration and congestion of blood vessels. Gills showed secondary lamellar disorganization, rupture in

lamellar epithelium and epithelial lifting.

Due to metal pollution, cellular level damage has been observed, which possibly affect the ecological balance. The toxicity of cadmium was tested in a toxicity test with rainbow trout fish, and it was found that cadmium had lesser intensity of toxicity in hard water (96 hr LC_{50}=2.6 mg Cd/L) than in soft water (96 hr LC_{50}=1.3 mg Cd/L). The industries pour wastes containing chromium and large amount of chromates, dichromate, and other chromium compounds into the aquatic system. The decreased levels of glycogen, protein and cholesterol have been observed after the administration of potassium dichromate in *L. rohita* (Madhuri et al., 2012a). Furthermore, chromium is present in the tannery effluent and causes various ill effects. Such health hazards are dependent on the oxidation state of chromium. The haematological changes produced on the exposure to sublethal concentration ($1/10^{th}$ of LC_{50}/96 hr) of chromium (VI) have been observed in freshwater fish, *L. rohita* for 7 and 30 days, respectively. The decrease in haematological parameters suggested that the exposed fishes became anaemic due to exposure of chromium. Hence, this heavy metal is severely toxic to fish when discharged via the effluents into the aquatic environments (Praveena et al., 2013).

8

TOXICITY OF MERCURY IN FISH

MERCURY EXPOSURE AND ACCUMULATION IN FISH

Much of the matters regarding exposure and accumulation of mercury and its cycles in fish have been discussed in Chapter 6. As mentioned earlier, mercury is widespread in the environment as a result of natural and anthropogenic releases. Most atmospheric mercury is elemental mercury vapour and inorganic mercury. The mercury present in waters, soils, plants and animals is typically present in organic or inorganic forms. Mercury is released into the surface waters from natural weathering of rocks and soils, and from volcanic activity. It is also released from human action, e.g., industrial activities, fossil fuel burning and disposal of consumer products (i.e., mercury thermometers, fluorescent bulbs and dental amalgams). The global cycling of mercury via air deposition occurs when mercury evaporates from the soils and surface waters to the atmosphere. From the atmosphere, the mercury is redistributed on land and surface waters; then absorbed by soils or sediments. Once the inorganic mercury is released into the environment, the bacteria convert it into organic mercury (e.g., methyl mercury), which is the primary form that accumulates in fish and shellfish (Pandey et al., 2012a). It is estimated that 50 to 70% of the total emission of mercury to the environment is a result of human activity. About 1,000 twice as much, about 2,600 tons of mercury is emitted from the anthropogenic sources (Honda et al., 2006).

Mercury is also found naturally in cinnabar, the major ore for the production of mercury. The anthropogenic sources of mercury vapour are emissions from coal-burning power plants, municipal incinerators and through the recycling of automobiles. Furthermore, mercury emitted from all sources is cycled through the ecosystem. Once in the atmosphere, the mercury vapour is slowly converted by oxidative processes to divalent mercury, which is then returned to the earth's surface by rainfall, where it accumulates in soils and in surface waters. Some of the mercury load is then converted

back into mercury vapour and returned to atmosphere. However, another fraction of mercury load is washed into rivers, streams and eventually the ocean, where it accumulates in the aquatic sediments. Then, the inorganic mercury is converted to methyl mercury by microorganisms living in the sediments by a process called *'methylation'* (Fig. 43). The methyl mercury then enters the *'food chain'* where it is absorbed by phytoplankton species. The phytoplanktons are eaten by plankton consumers, which then are eaten by larger fish and larger mammals (Fig. 44). The methyl mercury accumulates in the tissues of fish and shellfish via *'biomagnification'*, through which methyl mercury concentration increases as it moves up from one trophic level to the next. Within each organism, methyl mercury bioaccumulates as the organism consumes more and more organisms containing methyl mercury. Thus, smaller fish that are lower down in the food chain have lower concentrations of mercury in their tissues; while larger fish that are higher up in the food chain have higher concentrations. For example, sardines contain about 0.01 ppm of mercury, while sharks contain from 1 to 4 ppm. Fish with highest levels of mercury are sharks, swordfish and king mackerels. Large marine mammals like whales have levels similar to these fish. In the aquatic food chain, methyl mercury biomagnifies as it is passed from lower to higher trophic levels through the consumption of prey organisms. The fish at the top of the food chain can biomagnify methyl mercury approximately 1 to 10 million times greater than the concentrations in the surrounding waters. About all of mercury found in the fish and other aquatic organisms are in the methyl mercury form. The long-lived predatory ocean fish may have increased methyl mercury content because of the exposure to natural and industrial sources of mercury (Clarkson and Magos, 2006; Pandey et al., 2012a).

Fish accumulate substantial concentrations of mercury in their tissues, and thus can represent a major dietary source of mercury for human. Thus, fish are the single largest sources of mercury for human (Emami Khansari et al., 2005; Madhuri et al., 2012b). Methyl mercury accumulation in seafood and fish products is a growing global concern that poses severe health risks to the public. Mercury is a naturally occurring heavy metal and a waste product of industries such as coal-burning power plants. Once mercury enters the water, it is consumed by microorganisms, which are eaten by smaller fish, and these,

in turn, by bigger fish. At each step of food chain, mercury is retained in the muscle meat of fish, resulting in the highest concentrations of mercury in large, long-lived predatory fish, such as swordfish and shark (Pandey et al., 2012a).

TOXIC DOSES OF MERCURY FOR AQUATIC ANIMALS

The maximum allowable concentrations (limits) of different types of mercury compounds are as follows: elemental mercury- 0.1 mg/m³ in air; organic mercury- 0.05 mg/m³ in air; methyl mercury- 1 ppm (1 mg/L) in sea food; and inorganic mercury- 2 ppb (0.002 mg/L) in water (Wikipedia, 2013c). The toxic concentrations of mercury salts range from less than 0.1 µg/L to more than 200 µg/L for representative species of marine and freshwater organisms. The lethal concentrations of total mercury to aquatic fauna range from 0.1 to 2 µg/L (ppb) of medium. Several biological and abiotic factors modify the toxicity of mercury compounds- sometimes by an order of magnitude or more- but the mechanisms of action are not clear. Significant adverse sublethal effects were observed among selected aquatic species at water concentrations of 0.03 to 0.1 µg Hg/L (Eisler, 1987).

In a case report, a human victim was killed by 300 ppm of mercury. The mercury *'burn'* was seen on the skin of the fishes. It is estimated that over 60,000 foetuses will suffer from methyl mercury toxicity in the uteri from mothers eating swordfish, shark and tuna fish. The LD_{50} values of mercury are as low as 1 mg/kg in small animals (Dodd, 2013; Eck and Wilson, 1989).

Based on the growing body of evidence concerning the health issues of methyl mercury accumulation in the body, the EPA and *'Food and Drug Administration'* (FDA) of USA have issued advisories targeting consumption of fish for specific groups. Their advice to women who may become pregnant, pregnant women, nursing mothers and young children up to 6 years of age is to avoid certain types of fish high in methyl mercury, and limit the amount of fish consumed each week. Specifically, the EPA and FDA advise these groups not to eat shark, swordfish, king mackerel, or tilefish at all because they contain very high levels of mercury (>1 ppm). They also advise these groups to eat up to 12 ounces (or 2 average meals) a week of fish and shellfish that are

low in mercury. Children should only eat 6 ounces of fish. The low mercury fish and shellfish include shrimp, canned light tuna, pollock, salmon and tilapia. *Albacore tuna* is a commonly eaten fish but contains moderate amount of mercury. The EPA and FDA advise eating only 6 ounces of *A. tuna* for a week. Also, if one exceeds the suggested amount of fish or shellfish in a week, simply cut back the amount consumed the next week or two. Lastly, the EPA and FDA advise the public to check for local advisories on fish caught from local lakes, rivers and streams. These fish may be more greatly affected by anthropogenic pollution sources. These guidelines are not aimed at adult men, or woman past child bearing age, but individuals concerned with possible exposure to mercury should follow them as well. The current action level of US FDA for mercury in fish tissue is 1 ppm (Pandey et al., 2012a).

TOXIC MECHANISM OF MERCURY

Among the metals tested, mercury was found to be most toxic to aquatic organisms, and the organic mercury compounds showed greatest biocidal potential. In general, the mercury toxicity is higher at higher temperatures, at reduced salinities in marine organisms and in the presence of other metals like lead and zinc (Eisler, 1987).

Methyl mercury is 1,000 times more soluble in fat than in water, and it concentrates in muscle tissue, brain tissue and CNS. The mercury levels in fish may be in excess of 10,000 to 100,000 times the original concentration in surrounding waters. Its accumulation is fast, while depuration is extremely slow. The half-life of methyl mercury in fish is estimated at 2 years. The mercury levels for saltwater fish average 0.35 to 70.02 ppm (Burger, 2009; Madhuri et al., 2012b). The methyl mercury content of fish varies by species and size of the fish as well as harvest location. The top 10 commercial fish species (i.e., canned tuna, shrimp, pollock, salmon, cod, catfish, clams, flatfish, crabs and scallops) represent about 85% of the seafood market and contain a mean mercury level of about 0.1 μg/g (Pandey et al., 2012a).

As body tries to rid itself of these toxins, gaseous mercury is oxidized to divalent mercury, which accumulates in the kidney and can cause damage. Brain, kidney and lung are the target organs of elemental (gaseous) mercury. Brain and foetal brain are also the

target organs for methyl mercury (Honda et al., 2006). While some of the mercury compounds are fairly inert, many of them are extremely toxic. In the USA, some of the mercury-containing products have been banned, have limited use, or have special disposal requirements. These include dental fillings, vaccines, non-industrial thermometers, lamps, car starters and electronics. Most organic mercury compounds are readily absorbed by ingestion and appear in the lipid fraction of blood and brain tissue. The organic mercury readily crosses blood-brain-barrier (BBB) and placenta. The foetal blood mercury levels are equal to or higher than maternal levels (Pandey et al., 2012a).

Methyl mercury is easily absorbed in the digestive tract, where it forms a complex with amino acid cysteine. This new complex resembles a large neutral amino acid found in the body, methionine, and can more easily gain entry into cells. As with inorganic mercury, once in the bloodstream, methyl mercury will accumulate in the brain and cause damage to central nervous system (CNS). Methyl mercury is naturally removed from the body over time. Eventually, this methyl mercury-cysteine complex is transported to liver, where it is secreted into the bile, after which enzymes break the complex down into its amino acid and methyl mercury parts. Some amounts of this methyl mercury then come in contact with bacteria in the intestine and is broken down into the inorganic mercury and carbon. Inorganic mercury is poorly absorbed in rest of the methyl mercury that does not interact with bacteria is reabsorbed by body and goes through the process again. It takes about 30 to 40 hours for methyl mercury to be distributed to the tissues of body. This cycle is the reason it takes so long to rid the body of mercury and how it can accumulate in the blood. It can take up to a year for mercury levels to drop significantly (Clarkson and Magos, 2006; Pandey et al., 2012a).

MERCURY TOXICITY IN FISH

In the 1950's, one of the most severe incidents, known as the *'Minamata Bay Incident'*, of industrial pollution and *'mercury poisoning'* occurred in the small seaside town Minamata of Japan. A local petrochemical and plastics company, Chisso Corporation, dumped an estimated 27 tons of methyl mercury into the Minamata Bay over a period of 37 years. The mercury was used as a catalyst in the production of

acetaldehyde, a chemical employed in the production of plastics. The methyl mercury-contaminated waste water, a byproduct of the process, was pumped into the bay, creating a highly toxic environment that contaminated the local fishes. The residents of Minamata, who relied heavily on the fish for food, were at risk of exposure to methyl mercury with every bite of fish they ate. The high contamination levels in the people of Minamata led to severe neurological damage and killed more than 900 people. An estimated 2 million people from the area suffered health problems or were left permanently disabled from the contamination (McCurry, 2006). This form of toxicity in humans is now called *'Minamata disease'*. The symptoms of this disease include sensory disorders of 4 extremities, loss of feeling or numbness, cerebellar ataxia, tunnel vision or blindness, smell and hearing impairments, and disequilibrium syndrome. More serious cases lead to convulsions, seizures, paralysis and possibly death. In addition to the outbreak among the town people, *'congenital Minamata disease'* was seen in the babies born to the affected mothers. These babies showed the symptoms of cerebral palsy (Honda et al., 2006).

Mercury levels in bluefish are high enough to cause potential adverse effects in the sensitive birds and mammals that ate them and to provide a potential health risk to humans who consume them. Eating of fish larger than 50 cm fork length containing average levels of mercury above 0.3 ppm should be avoided by the pregnant women, children and others who are at risk. The communities that relied on fish intake for daily nutrient sustenance may be at risk from chronic, high exposure to methyl mercury, as well as other persistent organic pollutants. Similarly, high-end fish consumers, whether recreational or subsistence, are at risk from mercury exposure (Burger, 2009).

Many adverse health effects are associated with the accumulation of mercury in the body; though these vary depending on the amount of mercury one is exposed to, time of exposure, chemical form of mercury and age of the subject (Clarkson and Magos, 2006). Mercury is a well known environmental toxin. Its presence in the oceans, and therefore in the ocean fish, has had a disturbing effect on the public due to fear of biomagnification, or the progressive transfer of toxins up the food chain (Davis, 2010).

Accumulation of mercury in fish may harm the fish and other animals that consume

them. The birds and mammals that eat fish are more exposed to mercury than other animals which live in the aquatic ecosystems. Toxic effects of mercury on wildlife, including fish are reduced fertility, kidney damage, slower growth and development, abnormal behaviour and even death. Whales and dolphins may also be at high risk from mercury exposure (Wikipedia, 2013c). High toxic levels of mercury cause convulsion, anorexia, tremor, swollen gum and behavioural disturbances in animals (Madhuri et al., 2012a). The organic mercury compounds are most toxic to CNS, and may also affect kidney and immune system. The methyl mercury is toxic to cerebral cortex and cerebellum in the developing brain and is a known teratogen (Pandey et al., 2012a).

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ABOUT THE AUTHOR

Prof. Govind Pandey, having about 33 yr. of experience in 'Research/Teaching/ Administration/Extension', is an able academician, scientist, veterinarian and administrator; a Hindi literalist and eloquent speaker endowed with strong writing flair. Dr. Pandey is probably "only person in Madhya Pradesh and alone veterinarian in India with maximum academic qualifications" (20 Degrees/Diplomas/Certificates). He obtained PhD (Hons.) in Veterinary Pharmacology &Toxicology from JNKVV, Jabalpur in 1990. His "Biography" is included in the famous book, "Who's Who in the World 2011" (28th edition, America).

During his career started from 7th August 1980, Dr. Pandey worked in several capacities like "Veterinary Assistant Surgeon/Lecturer/Veterinary Surgeon/Senior Veterinary Surgeon/Drawing & Disbursing Officer (DDO)" under Animal Husbandry Department, Govt. of MP; "Chief Executive Officer/Block Development Officer/DDO" of some Janapad Panchayats under Panchayat & Rural Development Department, Govt. of MP; and "Assistant Professor/Professor/ Principal Scientist & Head" of Pharmacology in Pharmacy colleges. Besides, he served as "Deputy Director/Associate Professor/Senior Scientist" of Research Services, Nanaji Deshmukh Veterinary Science University (NDVSU), Jabalpur from 20th April, 2012 till he resumed the post of "Professor/Principal Scientist & Head", Department of Pharmacology & Toxicology, College of Veterinary Science & AH, Rewa (NDVSU, Jabalpur) on 26th November, 2012. Dr. Pandey is working in different areas of Life Sciences, with specialization in Pharmacology & Toxicology. He has also made a good contribution in Fishery Science, Hindi literature, HRM, Political Science, Sociology, Public Administration, Law and Astrology. He has investigated some "antihepatotoxic and anticancer herbal drugs, oestrogen induced cancer model, and ipomeamarone and paracetamol induced hepatotoxic model". Researches on 'oestrogen' (ethinyl oestradiol, an oral contraceptive and hormonal replacement therapy used by millions of women) induced cancer and its treatment, and 'ipomeamarone' (isolated from injured *Ipomea batatas,* sweet potato tuber) induced hepatotoxicity were successfully done for the first time in India. These researches were widely acclaimed by eminent personalities, scientific societies and media.

For great contribution in science/research/Hindi literature, Dr. Pandey has received "30 Awards/Honours". His PhD work on 'indigenous hepatogenic drugs' was adjudged as the outstanding research work, for which he received "Sri Ram Lal Agrawal National Award" (1991). He also received "ICAR Senior Research Fellowship (1986)" and many "Best Paper Awards". He has been honoured as the "Fellow of Academy of Sciences for Animal Welfare (FASAW)", "Fellow of Society of Life Sciences (FSLSc)" and "Fellow of International Science Congress Association (FISCA)". Prof. Pandey has guided many research scholars and carried out some projects. In science/research, he has authored "6 e-Books/e-Manuals" (all published from ISCA, Indore; 2013) and edited "1 Manual". He has published about "225 Papers", including "3 Book Chapters", and presented several papers in many conferences. In Hindi literature, he has authored "5 Books", edited "1 Book", released 2 audiocassettes ("Tarang" and "Archan") of own lyrics and published/broadcasted several poems/lyrics/dramas/stories through different media. He has been the "Editor/Editorial Board Member/Reviewer" of many books/journals/papers/ magazines. He has also been the "Life Member" of about 25 scientific, professional, literary and cultural associations/societies/journals. He was "Chairperson/Chief Guest/Judge/Expert/Advisor" in many conferences/committees/programmes. Dr. Pandey had been the "Badminton Captain, NCC Sergeant, Literary Secretary and Hostel Prefect" at the College of Veterinary Science & AH, Jabalpur, and passed "NCC B & C Certificates" and "2 years' Course of NSS".