Chapter

Health Hazards of Toxic and Essential Heavy Metals from the Poultry Waste on Human and Aquatic Organisms

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Abstract

This research was conducted to examine the impact of some essential heavy metals used as a supplement during animal feed formulation and the toxic from unregulated discharges of untreated poultry waste into water bodies on man and aquatic organisms. During the processing of poultry feed, certain heavy metals are used as a supplement such as selenium, copper, zinc, iron etc. to enhance poultry meat and egg yield which is also increase the daily discharge of anthropogenic wastes into our environment that contain high concentration of heavy metals discharges into aquatic environment globally, especially in underdeveloped where this waste are not treated before discharge or used in agriculture as an organic fertilizer in planting crops as a result of this it become absorb by plants and could pose a serious health risk to man and aquatic species as well as affect the ecological balance that can be transfer to humans via the food chain. Some organisms are kills as a result of the toxic heavy metals in water and can affect their growths. Bio-accumulated in the body of certain species, such as fish, which are eaten by humans that causes devastating diseases such as Minamata and Itai-Itai. Regulation of the use some heavy metals as a supplement in feed production or complete removal of it in animal feed should be adopt in order to minimize the human health risks and environmental contamination associated with these animal waste.

Keywords: essential heavy metals, animals feed, health risk, supplement, growth, toxic metals

1. Introduction

Water covers about 70 per cent of the Earth’s surface, makes up about 75 per cent of human body mass, and is the basic material that all living things need to live. The fact that water covers more than two-thirds of the Earth's surface makes it hard to believe that it is a scarce resource and that less than 1% of the total water on this planet is readily accessible for drinking or other uses. Approximately 97% of the earth's water is salt water contained in lakes or seas; just 3% is fresh water. However, 68 per cent of freshwater on Earth is enclosed in the Antarctic and Greenland ice caps (30%) while just 0.3 per cent is enclosed in surface waters, including lakes, rivers, reservoirs, springs and streams. Water
quality can be defined by its physical, chemical, biological and esthetic characteristics (appearance and smell) as well as by its fitness for the beneficial uses it has in the past provided for human and animal drinking, for the promotion of a healthy aquatic life, for irrigation of the land and for recreation. A safe water ecosystem is when it meets the standard in term of water maintains a rich, diverse population of species and is conducive for the consumption of public health. Water is of course, the basic liquid medium for living matter; thus, it is uniquely vulnerable to contamination by living creatures, including those that cause disease to humans. Aquatic contamination occurs as a result of the introduction by humans of either direct discharges into the water body or indirect substances or/energy that may result in the degradation of the water quality of any water body that poses a danger to human health, harms living organisms and hinders aquatic activities such as fishing and polluted water quality with respect to its use. Contamination mechanisms including suspension, solution and biochemical alteration is not inherently separate and distinct from each other and all of these complex processes may only occur in water. However, growing anthropogenic activities, such as urbanization, Industries, agricultural waste, etc. and natural processes, reduce water quality and pose a danger to all modes of life. Most people live in underdeveloped countries still depend on unprotected/contaminated water sources as their primary sources of drinking water and at the same time, as their means of waste disposal, which can cause outbreaks of waterborne diseases. The discharge of industrial waste into water bodies constitutes approximately 62 per cent of the overall source of heavy metals such as lead (Pb), zinc (Zn), copper (Cu), nickel (Ni), cadmium (Cd) and chromium (Cr) [1]. It is important to write about contamination caused by heavy trace elements, since untreated waste materials discharged by industry or agriculture worldwide are very concerned about the current disposal of waste materials containing heavy metals such as mercury, cadmium, lead, copper and arsenic due to growing concentrations in many waters.

2. Poultry farm waste

Poultry farms are one of the world’s leading sources of high-grade and palatable protein-rich food (eggs and meat) but domestic, industrial and agricultural poultry waste is regularly disposed of without treatment into water bodies, especially in most developing countries. Poultry farming is a lucrative global trade in animal husbandry that raises domesticated birds such as chickens, ducks, quails, pigeons, guinea fowl, turkeys and geese to produce meat or eggs for food originating in the agricultural period. According to the World Watch Institute, 74% of meat consumed worldwide is from poultry meat, and 68% of eggs are derived intensively from poultry, while more than 60 billion chickens are killed annually for consumption [2]. There is little doubt that the demand and therefore the production of poultry will continue to increase relative to the world population, the economy and also the increase in the production of poultry wastes. Poultry waste is used as manure in many fields, but when disposed of in a water body without treatment, it may cause significant problems for aquatic life due to the presence of heavy metals in it. Poultry waste as a mixture of different media involving feces, bedding materials, wasted feeds and feathers, represent favorable media for wide range of chemical and biological hazards include many food-borne pathogens like Salmonella, Campylobacter, Listeria, Actinomycets, Escherichia coli and Clostridium at high concentrations could reach up to $10^{10}$ CFU/gram [3].
Due to increased demand for livestock meats and eggs, there is also a need for increased use of trace elements (some of which are also heavy metals) as nutritional supplements in poultry diets to boost feed quality, promote weight gain and prevent disease, resulting in increased concentration of trace elements added to poultry diets. However, poultry feeds, whether natural or locally sourced or improved by special manufacturing processes, have been reported to be affected by the content of heavy metals in the feed [4]. Many heavy metals are also added to poultry feed as supplements, including copper (Cu), manganese (Mn), iron (Fe), selenium (Se), zinc (Zn) which are important nutrients needed for various biochemical and physiological functions in species, and a lack of supply of these micro-nutrients results in a number of deficiency diseases or syndromes [5]. Iron and Cu are added to prevent anemia, selenium is added to prevent oxidative cell damage, and Zn and Mn are added to ensure proper egg shell deposition and feather growth [5]. Calcium (Ca$^{2+}$) is added for bone formation, while in mature laying fowl the majority of dietary calcium is used for egg formation and plays a role in blood clotting and intracellular communication. Antioxidants are added to delay the deterioration of vitamins in poultry feed and tranquilizers may be used to keep flocks quiet in the house and during transport to another pen. A wide variety of antimicrobial drugs are commonly administered to poultry feed as prophylaxis and/or growth promoter and most of the oral applied antibiotics are poorly absorbed in the poultry gut, and then consequently those large amounts of antibiotics were excreted in feces and urine to the environment. Approximately 90% of the applied antibiotics might be excreted as the parent compound [6]. The most common antibiotics such as bactracin, chlortetracycline, monesin, tylosin, penicillin, chloramphenicol and virginiamycin can be applied to poultry feed to fight diseases, pests and increase the

| Trace/heavy metals | FAO/WHO [7] and EU [8–11] | National Research council [12] |
|--------------------|---------------------------|--------------------------------|
|                    | Metals requirement in total diet dry (mg/kg) | Metals requirement in normal diet (ppm) | Toxic level in total diet (ppm) |
| Cadmium            | 1 mg/kg                    |                                |                              |
| Chromium           | 0.01 mg/kg                 |                                |                              |
| Cobalt             | 1 mg/kg                    |                                |                              |
| Copper             | 100 mg/kg                  | 6–8 ppm                        | 250–800 ppm                   |
| Iron               | 45–80 mg/kg                | 50–80 ppm                      | 4,500 ppm                     |
| Iodine             |                            | 0.3–0.4 ppm                    | 625 ppm                       |
| Lead               | 1–5 mg/kg                  |                                |                              |
| Manganese          | 20–60 mg/kg                |                                |                              |
| Molybdenum         |                            | 3–5 ppm                        | 20–10 ppm                     |
| Mercury            | 0.5 mg/kg                  |                                |                              |
| Nickel             | 0.05 mg/kg                 |                                |                              |
| Selenium           |                            | 5–20 ppm                       |                               |
| Zinc               | 600 mg/kg                  | 40–75 ppm                      | 800–4,000 ppm                 |

Table 1. Permissible limits of trace/heavy metals requirements as an additive in poultry feed.
Trace mineral requirements for chicken (per kg of finished feed) mineral.

| Trace mineral | Egg Layer | Broiler | Broiler Breeder | Cockerel |
|---------------|-----------|---------|-----------------|----------|
|               | Chick Mash (0–8 Weeks) | Grower Mash (9–17 Weeks) | Layer 1 (18–45 Weeks) | Layer 2 (46–72 Weeks) | Pre-Starter (0–8 Days) | Starter (9–21 Days) | Finisher (22–42 Days) | Breeder Starter (0–8 Weeks) | Breeder Grower (9–17 Weeks) | Breeder Female 1 (18–45 Weeks) | Breeder Female 2 (46–72 Weeks) | Cockerel Starter (0–8 Weeks) | Cockerel Finisher (9 Weeks – Market) |
| Manganese (mg) | 60 | 60 | 60 | 60 | 30 | 30 | 60 | 60 | 90 | 90 | 60 | 60 | 60 | 60 |
| Iron (mg) | 30 | 30 | 30 | 30 | 80 | 60 | 60 | 30 | 30 | 30 | 30 | 30 | 30 | 30 |
| Copper (mg) | 6 | 6 | 6 | 6 | 5 | 4 | 4 | 6 | 6 | 12 | 12 | 6 | 6 | |
| Zinc (mg) | 60 | 60 | 60 | 60 | 40 | 35 | 35 | 60 | 60 | 100 | 100 | 60 | 60 | |
| Iodine (mg) | 0.5 | 0.5 | 0.5 | 0.5 | 0.4 | 0.4 | 0.4 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 | 0.5 |
| Selenium (mg) | 0.3 | 0.3 | 0.3 | 0.3 | 0.2 | 0.2 | 0.1 | 0.1 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 | 0.3 |

Source: SON [12].
supply of certain nutrients that transferred through the food chain to humans that induce antimicrobial resistance in humans. Topical pesticides are used as a repellent against flies, lice, bugs, mice and reptiles that can harm or destroy them. WHO/FAO [7], NRC [12], EU [8–11] and SON [13] set acceptable levels of metals in animals, but excessive or deficient use of these metals may lead to deformity in the body or to health problems, some of which may cause serious toxicity, which may lead to the death of the animal (Tables 1 and 2). However, pollutants from poultry waste can have detrimental environmental consequences (air, soil and water) if their waste is poorly handled or untreated prior to disposal in the aquatic setting. The disposal of waste produced by the poultry industry is a long-standing concern due to the contribution of nutrients or as a source of heavy metal contamination to our water bodies. Livestock manure may be used as fertilizer in the agricultural sector, it may also degrade the quality of the environment, especially surface and ground water, if it is not properly managed [14]. Untreated poultry waste can degrade water quality when discharged directly to surface water by runoff. The key environmental and health threats associated with animal waste are the introduction of toxins into water sources, such as nutrient limitation (nitrogen and phosphorus), organic matter, sediments, bacteria and heavy metals, which have harmful effects on the living organism and change the nature of the water. However, all mineral elements, whether considered to be necessary or potentially harmful, can have an adverse food impact on humans and animals if they are included in the diet at an overly high concentration [15]. Trace mineral bioavailability is characterized as the proportion of the component consumed that is used for biochemical or physiological purposes [16]. In order to have a high bioavailability, the mineral component must be readily absorbed and rapidly integrate by the body. Bioavailability is mainly influenced by the chemical form of the mineral or the amount in the diet, the amount in the body of the animal, the concentration of other minerals in the diet, its age and the physiological state of the animal to which it is fed. The risk lies in the accumulation of manure-borne metals, as they are not biodegradable and ultimately become phytotoxic, and the long-term use of poultry waste on the soil could lead to the accumulation of heavy/trace elements that increase the potential bioavailability and toxicity of metals in the environment. Such accumulation has the potential to limit soil function, contaminate water and cause toxicity to plants, animals and humans via the food chain. Their bioavailability is determined by physical, chemical and biological factors such as temperature, adsorption, sequestration, lipid solubility and water partition coefficients, whereas biological factors such as species characteristics, trophic interactions and biochemical/physiological adaptation also play an important role [17]. Poultry waste is more toxic than other animal waste due to the high concentration of heavy metals in poultry feed which is not directly absorbed by the body of the animal and egestion as a waste product, while the land application of poultry manure may result in the absorptions of toxicants by plants, animals and humans through absorption, ingestion, bioaccumulation or other processes.

4. Heavy/or trace metals

The word heavy/or trace metals is sometimes used loosely, as they contain a number of metals, some of which are not heavy and some of which are not metals. Heavy metals are a wide class of inorganic chemicals that are harmful to both human and environmental health. Heavy metals are commonly referred to as metals with a minimum density of more than 5 gm/cm3 and adversely impacting the environment and living organisms. Heavy metals include all metals and metalloids except alkali and alkaline earth elements. Some heavy metals are necessary for enzymatic
activity and can inhibit enzyme activity when natural concentrations are exceeded. Although some heavy metals are needed as micronutrients, they may be toxic at higher levels than their requirements. In addition, elements such as C, H, O, N, P, S, K, Ca and Mg are often required by majority of species in very small amounts. These elements are called trace elements, such as Fe, Mn, Cu, Co and Mo, and are usually considered to be necessary for most organisms, although V, B and Zn are confirmed to be essential in at least some cases. Most of these trace elements function in an enzyme or in an active group in an enzyme. Since heavy metals cannot be degraded, they are deposited, assimilated or incorporated into water, soil and marine organisms, causing heavy metal contamination in water bodies. Essentials include iron, copper, zinc, cobalt, manganese, chromium, molybdenum, selenium, tin, nickel and vanadium. The deficiency or elevation of these elements can affect the body’s normal physiological activities and biochemical processes, resulting in abnormal cell metabolism, development, reproductive disorder and severe oxidative. Non-essential metals are lead, cadmium and mercury. Cobalt, copper, chromium, iron, manganese, nickel, molybdenum, selenium, tin and zinc, sometimes known as trace metals. As a result, the majority of heavy metals, whether necessary or not, are potentially harmful to all living organisms, depends on many factors, such as dosage intake, species chemical composition, age of organisms, gender, genetic make-up and nutritional status of exposed individuals [17]. They have various effects on species depending on dosage exposure and durations of consumption: acute poisoning occurs when exposed to high doses over a short period of time, and chronic poisoning or bioaccumulation occurs when exposed to low doses over a long period of time.

‘Toxic metals, including ‘heavy metals, ‘are individual metals and metal products that have harmful human health effects either by direct or indirect exposure. Trace minerals or heavy metals used in animal feed are often expressed either as parts per million (ppm) or as milligrams per kilogram (mg/kg) of dietary dry matter. In very small quantities, many of these metals are required to sustain life and become toxic in large quantities. They can build ups in biological systems and become a major health hazard” [18]. The term heavy metal refers to any metallic chemical elements that have a comparatively high densities compared to water and are found in traces in different matrices. Their heaviness and toxicity are interrelated as heavy metals are capable of causing toxic or toxic at low concentrations and, if present in animal feed, pose significant health hazards to poultry meat consumers due to biomagnification effects in the body of the animal [19–22]. Heavy metals are normal components of the earth’s crust that are not depleted or damaged in the atmosphere and are harmful to human health because they appear to be bioaccumulate for a long period of time, e.g. mercury (Hg), cadmium (Cd), arsenic (As), chromium (Cr), thallium (Tl) and lead (Pb). Bioaccumulation refers to the rise in the concentration of the chemical in the body of the organism over time as opposed to the chemical concentration in the atmospheres. Accumulation of compounds in the organism at any time taken up is processed faster than broken down (metabolized) or excreted. Toxicity could result from any heavy metal, but ten (10) of them are among the top twenty hazardous substances considered to be toxic by several agencies due to their health implications, including arsenic, cadmium, chromium, cobalt, copper, iron, lead, manganese, mercury and platinum [17, 23]. In recent decades, the levels of these metals have risen in our environments as a result of human inputs and activities [24–26]. There are 35 different metals that are of considerable concern to human health due to residential or industrial exposure. They are widely present in the environment and animal diet as a food supplement and are needed in small quantities to maintain good health, but in larger amounts they become harmful or unsafe due to their accumulation in the animal’s body over time and may cause serious illness or death. Considering the great variety of heavy metals in the environment,
their concentration in various feed chains, it is difficult to achieve a lower level of toxicity than the detection limit for all elements in all products [27]. The European Union, the United States, Asia and other countries are aware of all these problems and as a result numerous laws have been implemented to regulate all heavy metal contamination, reduce the risk of human exposure in the food chain and develop detection methods to control these pollutants in the food chain [28].

**The most popular toxic heavy metals are the following:** Arsenic (As), Lead (Pb), Mercury (Hg), Cadmium (Cd), Nickel (Ni) and Iron (Fe).

### 4.1 Arsenic (As)

Arsenic is used in poultry production for growth promotion and for controlling intestinal parasites in which they are fed with arsenic compound called roxarsone (3-nitro-4-hydroxyphenylarsonic acid) while three-quarters of arsenic in feed will be excreted out as poultry waste into environment [29]. Arsenic is a natural soil constituents with concentrations of up to 500 mg/kg. In its essential form, arsenic is insoluble in water, but many of the arsenates are highly soluble. Much if not all-natural water contains compounds of arsenic. Arsenic is the most common cause of acute heavy metal poisoning in adults and is number 1 in the Top 20 List of ATSDR. Arsenic can also be present in water sources worldwide, contributing to contamination of shellfish, cod and haddock. The target organs are the blood, kidney, central nervous, digestive and skin systems [30]. Arsenic is noted for its human toxicity when ingestion of as little as 100 mg typically results in serious poisoning and 130 mg has been shown to be fatal [31]. Several incidents have shown that arsenic in water can be carcinogenic, that skin and probably liver cancers are due to arsenic in drinking water [32, 33].

### 4.2 Lead (Pb)

Lead is number 2 on the “Top 20 List.” for the ATSDR. Lead accounts for most cases of pediatric heavy metal poisoning [30]. Goal organs are bone, brain, blood, kidney, and thyroid gland [23, 34]. Some natural water contains as much as 0.8 mg/l of lead in solution [35]. These concentrations are also found in mountain streams that flow through limestone and galena. It causes acute and chronic toxicity and causes a wide variety of physiological, biochemical and behavioral dysfunctions in humans, animals and aquatic species. Addition of lead to the diet results in a dose-related rise in the concentration of Pb in different organs in the body of animals such as the kidney, blood stream, liver and tibia. It induces oxidative stress that suppresses growth efficiency and decreases feed intake and body weight loss.

### 4.3 Mercury (Hg)

The number 3 of ATSDR’s “Top 20 List” is mercury and naturally generated in the environment by degassing the earth's crust, by volcanic emissions [36]. It is available in three forms: elemental mercury, organic and inorganic mercury. Atmospheric mercury is spread across the globe by winds and returns to the planet in runoff, collecting in marine food chains and fish in lakes [37]. Many researchers believe that dental amalgam could be due to a source of mercury toxicity. Mercurochrome and merthiolate are still in use in drugs, while algaecides are the main possible sources of mercury by inhalation. The organic form is readily absorbed in the gastrointestinal tract (90–100%); Less but nevertheless large amounts of inorganic mercury are absorbed in gastrointestinal tract (7–15%) and the target organs are majorly brain and kidneys [30].
4.4 Cadmium (Cd)

Cadmium is a derivative from the smelting or mining activities of lead and zinc in environment and it occupied 7 position on ATSDR’s “Top 20 list.” It also used in nickel cadmium batteries production, PVC plastics, and paint pigments industries. It can also find in Cigarettes, as well as in soil as a result of insecticides, fungicides, sludge, and other commercial fertilizers that contain cadmium compound in agriculture or in reservoirs that contain shellfish. Other sources of cadmium contamination are from dental alloys, electroplating, engine oil and automobile exhaust. Inhalation of cadmium accounts for 15–50 per cent of assimilate into the respiratory tracts; 2–7 per cent of the ingested cadmium is absorbed into the gastrointestinal system while main target organs are the liver, placenta, kidneys, lungs, brain and bones [30]. Cadmium is moderately harmful to all species and is a cumulative toxin in mammals. In low concentrations, the use of trivalent chromium as an additive in animal diets may induce rapid growth for the animal in order to improve the quality of the meat produced, but often poultry owners may add trivalent chromium in excesses for rapid growth of their animals in order to obtain further value, which may have adverse effects on animals such as those injured and poisonous to the animal. It appears to be concentrated in the kidneys, liver, pancreas and thyroid of humans and other mammals. Humans can be exposed to this metal mainly through inhalation and ingestion, and can suffer from acute and chronic intoxication. Kar and Patra [38] reported that the Cd concentration sometimes increases in feeds, fodders, water bodies, and tissues of livestock which causes metabolic, structural, and functional changes of different organs of all animals. In poultry birds, bioaccumulation of Cd occurs in several organs mainly in the liver, kidney, lung, and reproductive organs due to its continuous exposure. Intake of Cd reduces growth and egg laying performance and feed conversion efficiency in poultry. Chronic exposure of Cd at low doses can also alter the microscopic structures of tissues, particularly in the liver, kidney, brain, pancreas, intestine, and reproductive organs due to increased contents of Cd in these tissues. Continuous Cd exposure causes increased oxidative stresses at cellular levels due to over-production of reactive oxygen species, exhausting antioxidant defense mechanisms. This leads to disruption of biologically relevant molecules, particularly nucleic acid, protein and lipid, and subsequently apoptosis, cell damage, and necrotic cell death. The histopathological changes in the liver, kidneys, and other organs are adversely reflected in hemogram and serum biochemical and enzyme activities.

4.5 Iron (Fe)

Iron does not appear on the ATSDR’s “Top 20 List, “ but it is a heavy metal of concerns, particularly because ingesting dietary iron supplements may acutely poison young children. Uses of Fe as additives in feed formation have many disadvantages such as low bioavailability, high hydroscopicity and oxidative, high excretion and so on [39]. Iron deficiency is still a major problem in several segments of the livestock production causes microcytic, hypochromic anemia in chickens. Iron also plays a role in other enzymes involved in oxygen transport and the oxidative process, including catalase, peroxidases, flavoprotein enzymes and cytochromes. Approximately two-thirds of body iron is found in hemoglobin (red blood cells and myoglobin in the muscles), while 20% is present in labile forms in the liver, spleen and other tissues, with the remainder not available in tissues such as myosin and actmysin and in metalloenzymes. The iron in hemoglobin is essential for the proper function of every organ and tissue of the body. The iron requirement of chicks fed casein, dextrose, and isolated soybean protein concentrate-based diet was studied.
by Aoyagi and Baker [40]. Ingestion accounts for most of the toxic effects of iron because iron is absorbed rapidly in the gastrointestinal tract and other target organs are the liver, cardiovascular system, and kidneys [30].

4.6 Zinc (Zn)

Zinc plays an important role in biological process in animal including immune function, growth, development and reproduction. It is component of many enzymes contributing in the energy metabolism, protein synthesis and degradation biosynthesis of nuclei acids, carbon dioxide, transport and many more. Its performance major role as an antioxidant in diet, growth and development, production, immunity and stress related issues. It is important in animal diets formation because it influences economic profitability of egg modifying. Zinc has a beneficial impacts on the growth and reproduction of livestock. Due to the low zinc and copper contents of some home-grown feeds compared to guidelines and varying bioavailability, supplementation of these metals is essential for most livestock species and is usually added as mineral supplements to dairy rations [7–9]. Zinc deficiency causes growth retardation and irregular production of feathers in poultry animals. Feather spattering occurs towards the end of the feather while severity of the spattering ranges from no feathers on the wings and tail to minor defects in the growth of some of the barbels and the hog joint may be widened. Zinc deficiency can cause the long bones of the legs and wings to be shortened and thickened. Other signs include loss of appetite, decreased feed use quality, and death in extreme cases. Zinc deficiencies in the breeding diet decreases egg production and hatchability. Embryos developed in zinc-deficient eggs display a wide range of skeletal anomalies in the head, limbs and vertebrae. The hatched chicks will also not stand, eat or drink [41]. Proper zinc supplementation has been shown to be effective in reducing the early mortality of poultry animals and zinc supplementation is typically applied to animal diets in the form of zinc oxide or zinc sulfate. Latest comparisons of bioavailability in chicks suggest that feed grade zinc oxide has just 44–78 per cent of zinc sulfate availability when added to refined or functional diets [42–44].

Zinc toxicities can cause health problems, and prolonged consumption can also lead to negative side effects such as nausea and vomiting, loss of appetite, diarrhea, abdominal cramping and immunity. The risk associated with zinc deficiency could cause gastrointestinal diseases such as Crohn's disease, decreased immunity, thinning of hair, decreased appetite, weight loss, skeletal malformations, poor bone mineralization, immunological dysfunction, mood disorders, dry skin, fertility problems and impaired wound healing, inadequate dietary intake, poor absorption, genetic mutations. Symptoms of extreme zinc deficiency include impaired growth and development, delayed sexual maturity, chronic diarrhea, impaired wound healing and behavioral problems [45, 46].

4.7 Nickel (Ni)

Nickel is an essential element required in low amount for animal growth and it is required for activities of vitamin B12 and biotin during metabolism of odd-chain fatty acids in animals [47]. Depending on the dose and length of exposure, as an immunotoxic and carcinogen agent, nickel can cause several health problems such as contact dermatitis, cardiovascular disease, asthma, lung fibrosis, and respiratory tract cancer [48, 49]. However, the exposure of human beings mainly concerns oral ingestion through water and food as nickel may be a contaminant in drinking water and/or food [50]. Although the molecular mechanisms of nickel-induced neurotoxicity are not yet clear, oxidative stress and mitochondrial dysfunction
have a significant role to play. Mitochondrial nickel-induced damage can occur due to impaired mitochondrial membrane potential, decreased mitochondrial ATP concentration and degradation of mitochondrial DNA [51]. Nickel, high concentrations of which can affect human health badly, can accumulate on plants, animals, and soil.

5. Other trace/heavy metals use as a supplement to prevent deficiency in poultry animals

**Copper** is necessary for the action of enzymes associated with the metabolism of iron, elastin and collagen formation, melanin production, and the integrity of the central nervous system [41]. Normal red blood cell formation is needed by enabling the absorption of iron from the small intestine and the release of iron into the blood plasmas in the tissue [41]. Copper is necessary for bone formation by promoting the structural integrity of bone collagen and the normal formation of elastin in the cardiovascular system. It needed normal myelination of brain cells and spinal cord as a component of the enzyme cytochrome oxidase, which is necessary for the formation of myelin. Maximum immune response also depends on copper, as shown by depressed titers in deficient animals [41]. The minimum requirement for copper cannot be provided with great precision, because the absorption and utilization of copper in animals can be significantly influenced by many mineral elements and other dietary factors. The process of natural hair and wool pigmentation includes the use of copper. Copper is believed to be a portion of polyphenyl oxidase that catalyzes the conversion of tyrosine to melanin and the incorporation of disulfide groups into keratin in wool and hair. Copper deficiency causes microcytic, hypochromic anemia, bone weakness, deformity and depigmentation in animals. In copper deficient chicks, aneurysm dissect the aorta, and in other species, cardiac hypertrophy occurs. Copper deficiency in laying hen causes anemia and the development of eggs that are abnormal in size and shape, and some eggs have wrinkled and rough shells [11].

**Molybdenum** is an essential nutrient because it is a constituent of the enzyme xanthine oxidase and other enzymes. When there is excess molybdenum in animal feeds it causes a copper deficiency which can results in extreme diarrhea, weight loss, bone and joint disorders, affect reproduction and heart function and anemia. Molybdenum has been shown to be essential for growth of animals such as lambs, chicks, and turkey fed highly purified diets. At this time, however, the Food and Drug Administration does not recognize molybdenum use as safe, and current regulations prohibit adding it to animal feed [52, 53].

**Manganese** was first recognized as a part of necessary nutrient required for growth of animals in the early 1930s, because it is found in many different animal feeds, the effect of its deficiency is less likely than with most of the other trace minerals. The highest manganese concentration can store in the body of animals such as in bone, kidney, liver, pancreas, and pituitary gland. Manganese deficiency in the diet of growing animas like chicks causes perosis, or slipped tendon but it deficient in chicks have less proteoglycan in the cartilage of the tibial growth which twisting and bending of the tibia, and slipping of the gastrocnemius tendon from its condyles [44]. With increase in severity, the chicks are reluctant to move, squat on their stools, and can lead to death of the animal. Lack of manganese breeding or in laying birds can lead to reduce in egg production, hatchability and reduced egg shell strength. In certain cases, most embryos that die due to manganese deficiency display chondrodystrophy which is a disorder characterized by a parrot-like beak, wire and shortening of long bones [41].
Iodine combined with tyrosine in the thyroid to form diiodotyrosine. Iodine deficiency in breeding hens could result in reduced egg iodine levels, reduced egg development, decreased hatchability, extended hatching period, and increased embryos in the thyroid gland. Goiter develops in the thyroid gland, which causes the thyroids gland to expand to several times its usual size. Histological analysis of the thyroid has indicated hyperplasia and lack of colloid. The thyroid gland contains the highest concentration (0.2 per cent to 5 per cent on a dry weight basis) of iodine in the body; 70 per cent to 80 per cent of the total body stocks. Approximately 90 percent of the iodine that passes through the thyroid gland is captured by that organ [54]. Two molecules of this compound are then mixed to form tyroxine. Approximately 80 per cent of the thyroxine entering the circulation is broken down by de-iodization of the liver, kidneys and other tissues.

Selenium is one of the most commonly known nutrient deficiencies in animal growth and was recognized as a potentially harmful mineral until it was identified as an essential nutrient. It plays a significant role in the preventing exudative diatheses in chicks and is present in all cells of the body while the concentration is generally less than 1 ppm but its harmful absorption in liver and kidneys are usually ranged between 5 and 10 ppm. Selenium is a crucial component of enzyme glutathione peroxidase that eliminate peroxide compounds from the body tissues and it essential in the synthesis of sulfur amino acids that helps in protecting animals from a variety of diseases associated with low intakes of selenium and vitamin E. Selenium and vitamin E are both effective antioxidants that prevent peroxide from destroying the body cells. Selenium can be added to the diet of all food animals. Birds may be fed up to 0.1 ppm of selenium in the total diet, whereas excess selenium in animal diets must be avoided if sufficient precautions are taken in addition to animal diets. All these animals need selenium at a level of 0.1 ppm in the total diets (except the turkey, which requires 0.2 ppm, and the baby pig, 0.3 ppm). Generally higher levels of protein, sulfur and arsenic can partly protect against the toxicity of excess selenium. Selenium is quickly extracted from the body of the infected animals when the animal is fed selenium-low [55]. In broilers feed with diet containing low selenium e.g., chickens of 3 to 6 weeks begin to display signs of weight loss, weakening of the leg and eventual lead to death. Extreme deficiency of selenium is shown with the sign of growth retardation and the mortality rate rise even in the presence of sufficient vitamin E. Disease like Pancreatic fibrosis, reduction of pancreatic output of lipase, chymotrypsinogen and trypsinogen are related to selenium deficiency. Pancreatic lesions disease arises as early as 6 days old chicken and typically return to normal within two weeks when selenium is used as a feed supplementation in their diet. The most sensitive requirements for selenium deficiency are egg hatchability in laying hens. Selenium results from encephalomalacia, membrane lipid peroxidation, erythrocyte hemolysis and muscular dystrophies [41].

6. Recent research on the quality of heavy metals in poultry feed

Based on the study by Eloma et al. [56], which analyzed six potentially toxic elements (PTEs) from poultry feeds such as Cd, Cr, Cu, Pb, Mn, Ni and Zn, four feed forms (starter, grower, finisher and layer) from four producers coded A, B, C and D were sold in Ebony State, Nigeria. The mean concentrations of metals recorded from poultry feeds were as following: Chromium (11.9–7.90 mg/kg); Copper (5.10–7.91 mg/kg); Cadmium (0.49–0.76 mg/kg); Lead (7.17–9.47 mg/kg); Manganese (26.9–34.9 mg/kg); Nickel (3.80–6.50 mg/kg) and Zinc (27.8–38.4 mg/kg). The result of these findings was compared with European Union standard of PTEs maximum
acceptable concentration in feed while Pb and Ni concentrations were above the maximum acceptable limits that is risk to human health. Thus, there is a need for continuous monitoring of feed compositions. Lead and Ni exceeded permissible limits by European Union in feed as stipulated, but the perilous elements such as Cr, Cu and Zn were also high in feed. There is however a need for continuous monitoring of feed compositions and also for the introduction of practices that will not introduce PTEs into the system. It also recommended that a proximate study be carried-out on poultry feeds to determine its moisture content, ash content, crude fiber, lipid, crude protein, carbohydrate and metabolizable energy [57–59].

Kabir and Bhuyan [60] were conducted to determine the heavy metal content of hens (Gallus gallus domesticus) and ducks (Anas platyrhynchos) from the Chittagong regions of Bangladesh. Chromium (Cr) and cadmium (Cd) concentrations were found below the detection limit in both hens and ducks of the egg. The concentrations of Iron (Fe) ranged from 58.4 to 78.90 mg/kg was recorded for hen and duck yolks and 3.90 to 11.62 mg/kg for albumin. The highest concentration was recorded in hen layer eggs (78.90 mg/kg), while the lowest concentration was observed in native duck eggs (58.4 mg/kg). The highest concentration of 11.62 mg/kg was recorded from albumin of indigenous duck eggs, while the lowest of 3.90 mg/kg was observed in indigenous hen eggs. The copper concentration ranged from 1.85 to 3.95 mg/kg was recorded from hen and duck yolk, while in albumin these amounts ranged from 0.25 to 1.15 mg/kg. The highest value (3.95 mg/kg) of indigenous hen eggs was reported, while the lowest concentration was 1.85 mg/kg for hen eggs. The highest concentration of 1.15 mg/kg was reported in albumin of domestic duck eggs, while the lowest value of 0.25 mg/kg was observed from native hen eggs. There was significant difference in the concentrations of Fe (p = 0.00) and Cu (p = 0.00) in both yolk and albumin. However, there were no major variations in the number of Fe (p = 0.998) and Cu (p = 0.458) in terms of the animal type (indigenous hen, indigenous duck, layer hen).

Korish and Attia [61] conducted research on heavy metal content in feed, litter, meat, meat products, liver and table eggs of chickens. Concentrations of heavy metals were examined in chicken meat, meat products, feed, litter, as well as laying hen eggs to track the regularity of this metals in the market products and their protection for human consumption as recommended daily allowance (RDA). Samples were collected from most popular poultry products in Saudi Arabia. A total of 45 samples from frozen broiler meat, fresh beef, liver, frankfurter and burger were collected from the same brand. However, 60 table eggs were collected from four different commercial brands while the edible parts of egg were analyzed to determine the levels of mineral elements present in it. In addition, 30 samples from different feed and litter were collected from the starter feed, grower feed, diets of layer broilers and laying hens. The findings showed that there were extensive amounts of most trace or heavy metals in the various meat sources while liver had the highest concentration of all elements examined, except for Co, Cr and Ni. The highest amount of Chromium concentration was recorded in fresh meat, followed by frozen meat. Trace or heavy metals such as Mn, Co, Ni and Pb were not detected in frozen or fresh meat. The chicken burger and the frankfurter samples have similar concentrations of trace/heavy metal except for Zn and Mn which had higher concentrations was observed in frankfurter compared to burger sample. There were significant differences between zinc concentration of the different sources of eggs. Fe was significantly higher in beef meat compared to poultry meat but the opposite trend for Zn was observed. All heavy metals concentration in were higher in liver than the eggs, except for Chromium while the burger had higher concentrations of Cu and Co. finally, it concluded that Cd, Pb, As and Se are not detected in chicken meat and eggs produced which indicate that no human hazards from these toxic elements.
However, the liver had the highest concentration of all heavy metals examined, except for Cr, and the intake of Pb and Cd from the broiler liver was higher than the RDA for adults. Burgers and frankfurters, showed higher concentrations of Pb, Cd and Ni than chicken meat and table eggs, implying a potential human health danger. Therefore, in order to enhance the quality of poultry products for human consumption, adequate legislation is required to regulate the quality of poultry products, as well as feed/food and chicken litter. In addition, critical measurements should be used for the detoxification of heavy metals from waste. The relationship between the minerals in poultry production and the diet of poultry and poultry litter remains fertile for further study.

Study of Dahri et al. [62] on the investigation of concentrations of heavy metals; lead and chromium in chicken feed collected from commercial poultry feed markets and local poultry farms in Hyderabad Sindh. A total of eight samples of poultry feeds, four of which were commercial feed samples and four of which were local feed samples collected in polyethylene bags. The samples were analyzed using the Aurora Al1200 Atomic Absorption Spectrophotometer (AAS) for heavy metals; lead (Pb) and chromium (Cr). Relatively higher concentrations of lead (Pb) have been found in commercial feed samples. Data obtained from the present study for lead and chromium beyond the allowable limit, i.e., 0.05 and 0.1 ppm as recommended by WHO/FAO. Lead (Pb) and chromium (Cr) metals are important for the growth of poultry, but they may become toxic if the concentrations exceed the allowable limits. Excessive quantities of metals taken by animals make their way to the human body, which is extremely dangerous to human health. Heavy metal contamination is prevalent in the Hyderabad district and thus in the present report, the amount of poultry feed is alarming. The nutritional values of the feed are therefore calculated from the concentrations of lead and chromium above the allowable level in the feed content.

7. Causes of heavy metal contamination in the body of water

Aquatic ecosystems are highly complex, diverse and subject to a variety of internal and external relationships that are subject to change over time. Public health issues are among the pollutants that the concentration of heavy metals in marine environments enters humans through food chains. Heavy metal contamination may occur from many causes, but most generally results from metal purification, e.g., copper smelting and nuclear fuel preparation. Following the introduction of heavy metal pollutants into the flow, whether from natural or anthropogenic sources, they divide between aqueous (pore water and overlying water) and solid phases (sediment, suspended particulate matter and biota). Anthropogenic metals can persistently persist inside water bodies, or these elements are absorbed by silt, likely absorbed by animals, and accumulate in the food chain, beginning easily with plankton, such as filtering zooplankton, benthos, or fish, and eventually transferred to humans. Unlike organic contaminants that lose biodegradation toxicity, heavy metals cannot be degraded/decayed and thus pose a different form of remediation challenge. Heavy metals such as lead, mercury, iron, cadmium, aluminum and magnesium are found in water supplies. If these metals are found in the sediment, they enter the food chain through plants and aquatic animals. Impact of heavy metals on aquatic organisms.

The effects of heavy metals on marine species vary from a small drop in the rate of growth to death. Pollutants entering inshore waters and estuaries cause severe problems, causing significant harm to the life and activities of living aquatic species and also to the mass mortality of organisms. The gradual and irreversible
accumulation of these metals in the various organs of the creatures of life contributes to long-term metal-related diseases due to their toxicity, endangering aquatic biota and other organisms [36]. Heavy metal pollution may have detrimental effects on the ecological balance of the recipient ecosystem and the diversity of marine species [63–65]. Among the animal species, fish are those that cannot avoid the adverse effects of these contaminants [66–68]. These metals are responsible not only for the deterioration of the water quality of the body, but also for the death of a variety of aquatic species [69]. The disposal of these wastes adversely affects water bodies, changes their chemical composition and causes harm to both humans and aquatic organisms [70–72]. These heavy metals (arsenic, cadmium, chromium, cobalt, copper, iron, lead, manganese, mercury and platinum) become toxic by accumulations of flora and fauna in the body tissues and then move through the food chain from fish to humans [73, 74]. Cadmium causes certain problems similar to those caused by mercury, which are much more harmful than mercury; Daphnia, Scenedesmus and E. coli are very susceptible [69]. Some of these heavy metals are important to human metabolism while some are dangerous, particularly when their concentrations are higher [75, 76]. The presences of heavy metals observed in most of marine animals is becoming a threats to human health, rendering them unfit for human consumption [1]. The presences of lethal metals in ecological environments are one of the key concerns of pollution control and environmental interventions in most parts of the world [77, 78]. Copper is more widely recorded as an algal toxin in contaminated waters than as a restricting algal growth [79]. Freshwater animals are probably more susceptible based on the estimates that 50 per cent of the waterfowl population of Daphnia magna died at concentrations between 25 and 65 μg/l, the exact values depending on the experimental exposure period and age of the animals. Ionic copper tends to be a real toxic material, whereas oxides or other colloidal particles or chelates are much less dangerous. In general, mollusks and fish tolerate higher concentrations of trace metals than other phyla studied. Acute and sub-lethal effects of zinc pollution on aquatic ecosystems have been extensively studied among different species of fish [80]. The toxicity of zinc to fish has been shown to depend on the nature of the water. Acute toxicity is increased by the rise in water temperature and the resulting reduction in oxygen content.

Mercury emission experiments in aquatic environments indicate that recovery from pollution will take place within a limited period of time following the cessation of pollution input [81]. The embryonic and larval stages of marine organisms are typically the most susceptible periods of the life cycle for heavy metals and other toxicants. Copper accelerated the mortality of Mytilus edulis during the reproductive cycle and impaired respiration by destroying the respiratory membranes. Mercury has often been seen with curiosity and alarm. It's the only metal that's liquid at a regular temperature (hence its other name, quicksilver) and it fun to play with. Its vapor is toxic and can vaporize quickly enough to be lethal at high temperatures. Any of its compounds, the toxicity of which has been well known since the Middle Ages, have been used as agents of murder and suicide. However, until quite recently, mercury was not considered a dangerous water pollutant, since it is harmful in vapor form and is not especially hazardous when taken by mouth as a liquid. Arsenic, lead, cadmium and mercury are cumulative cell poisons with a strong propensities to be deposited in the bone, particularly in the case of lead. The signs of moderate chronic poisoning are not well described and therefore difficult to diagnose. Heavy metals become harmful to the body of an organism when they are not metabolized and accumulate in soft tissues and can reach the human body through food chain, water, air, or absorption through the skin when they come into contact with humans [36].
8. Health hazards pose by poultry waste in an environment

The great concern lies in the excessive accumulation of macro-minerals (Ca, Si, Fe), trace elements (Cu, Mn, Zn, Se), heavy metals (Pb, Hg, Cd), medicinal drugs (antibiotics, coccidiostatics, sulfa drugs, etc.), anti-metabolites, insecticides, herbicides, wood preservatives, mycotoxins and hormones, harmful organisms transmittable other non-nutritional excretory via wastes to man. Poultry waste contains considerable amounts of nutrients (nitrogen and phosphorus) and other excreted substances such as hormones, antibiotics, harmful pathogens and heavy metals. Leaching and runoff of these substances has the potential to contamination both the surface water and/or nearby groundwater (Steinfeld et al., in [82]). Thus, increased outputs of phosphorus and nitrate to fresh water which can caused severe water quality problems like accelerate eutrophication in surface waters due to high inputs of organic substances and nutrients through runoffs which can result into accumulation pollution nutrient-sensitive ecosystems resulting in biodiversity losses such as fish kills due to hypoxia/anoxia and high levels of ammonia, harmful toxic algal blooms, decreases in water clarity, widespread anoxia, declines in submerged aquatic vegetation, shifts in pH, and depletion of oxygen. A drop in the level of dissolved oxygen in surface water has deleterious effects on fish populations [83, 84]. Furthermore, eutrophication can spur the growth of toxic microorganisms, such as *Pfiesteria piscicida*, that have been found to cause temporary memory loss, immuno-suppressions, and decreased cognitive function in exposed populations, respiratory problems and eye irritation, as well as gastroenteritis, headaches, and fatigue [6]. Skin irritation and lesions have also been reported among those with direct contact with contaminated surface waters, particularly among fishermen [6]. The leaching of nitrates and pathogens into water can cause significant cognitive loss and nervous system impairment when ingested by humans [6].

Poultry waste contains toxic metals that are bioaccumulate in the body of aquatic organisms and become biomagnification through food chain to next trophic level which can cause health hazard to human such as arsenic which is carcinogen and may also lead to heart disease, diabetes, and a decline in mental functioning. These harmful bacteria and chemicals present in poultry waste threaten the human health and aquatic organisms globally. Also of concern is the issue of air quality affected by dust particles, releases significant emissions of gases (methane, hydrogen sulfides, sulfur dioxide and ammonia) offensive odors and other pollutants such as volatile organic compounds (VOCs), particulate matter (PM), nitrogenous compounds during the decomposition of poultry waste that contributed to climate change which is a global concern and deleterious health effects (both chronic and acute) including respiratory conditions (i.e., bronchitis, asthma in children), heart disease, and lung cancer [85, 86]. Poultry waste as an important source of nutrients for many edible crops, may also contain some biological hazards that can threaten human health [87]. Poultry waste could be a source of human pathogens such as Salmonella, Campylobacter and Listeria that can potentially contaminate both edible crops and environment, which consequently leads to food-borne diseases [88, 89]. Poultry litter contains wide and diverse counts of microorganism including both of gram positive and negative bacteria. Among the bacterial and fungal species that are biological hazard to human health was recorded from poultry litter and waste such as *Actinobacillus*, *Bordetella*, *Campylobacter*, *Clostridium*, *Corynebacterium*, *E. coli*, *Globicatella*, *Listeria*, *Mycobacterium*, *Salmonella*, *Staphylococcus*, and *Streptococcus* [6]. Accumulation of high concentration of heavy metals initiates numerous fatal signs including hepato-renal dysfunctions and reproductive complications. Cd toxicity effect can persist more than 10–35 years in the body due to long biological half-life. After absorption, it is primarily stored and
distributed in various tissues, primarily, in the liver and kidney. It has been proved that Cd has direct toxic effects on the cellular levels which lead to apoptotic and necrotic cell deaths. It is also responsible for malignant growth and it is categorized as a type 1 carcinogen [38]. Exposure of Cd to livestock including poultry not only affects health, but also hampers animal production reducing growth performance and feed utilization efficiency in their body.

9. Diseases caused by heavy metals

Heavy metal contamination is known to cause numerous diseases worldwide, such as minamata disease (organic mercury poisoning), itai-itai disease (cadmium poisoning), arsenic acid poisoning, and asthma induced air pollution (Matsuo, 2003). The worst examples of accumulation are recorded in Japan, where hundreds of fishermen were killed by consuming fish containing too much mercury (Minamata Disease, [37]) or cadmium (itai-itai disease Kobayashi, 1971). These tragedies resulted from the tragic coincidence of a predominantly fish-eating and fish-eating population, which while having high concentrations of mercuries, did not show any symptoms. A special feature makes the mercury issue more important because under anaerobic conditions after sedimentation, it can be transformed to yet more toxic methylmercury compounds, which like metals, may accumulate in organisms that are possibly adsorbed to –SH groups in enzymes and even in food chains. Conversion to methylmercury is a bacteriological conversion involving methane bacteria. As a part of this reaction, mercury adsorbed to sediments may be mobilized after they are settled (which would also contribute to anaerobic conditions) and accumulate in fish via food chains. Copper is another aspect that creates concern in contaminated water. Approximately 1 g of copper causes acute illness in humans. Some freshwater algae tend to require either cobalt or vitamin B12. Copper is also less harmful than mercury, and incidents like Minamata are not likely to occur, nor do drinking water appear to be poisoning. Copper disposal is so common and widespread, however that amounts of copper in aquatic environments can typically be high enough to cause harm to species. If humans eat food tainted with heavy metals and their concentrations are amplified because they cannot be excreted. If the concentration exceeds a lethal level, it can results in brain injury or death. Lead affects the central and peripheral nervous systems, organs, bones and kidneys. Lead does not have an advantageous biological role and is believed to store in the body. Lead acquaintance can cause antagonistic effects on human health, particularly in young children and pregnant women, as Pb is a neurotoxin that always disrupts normal brain development. It accumulates in the skeleton, induces bone mobilization during pregnancy, lactation exposure to fetuses and breastfed babies. Cellular and molecular lead can increase the incidence of carcinogenic events associated with DNA damage and suppress DNA repair and tumor controls. Lead is a toxic metal that is particularly harmful to children. Health issues caused by low levels of chronic exposure to heavy metals may take years for humans to develop and may be related to heavy metals [36]. Most of the exposure to heavy metal contamination has been clinically shown to be associated with causing free radical harm leading to: heart attacks, strokes, cancer and several circulatory disorders other than cardiovascular diseases that do not cause death, but may affect the quality of life. Any of these include: Impotence, Asthma, Diabetes, Exhaustion, Alzheimer’s Disease, Memory Loss. Lead poisoning is a severe, very common form of heavy metal poisoning and the symptoms of lead poisoning in children are close to those of attention deficit hyperactivity disorder (ADHD). Lead poisoning also triggers behavioral and cognitive disabilities, nervousness, headaches, and many other associated symptoms.
Metals may be extracted from aqueous sources such as chemical precipitation, lime coagulation, ion exchange, reverse osmosis and solvent extraction [90]. Other methods include electrodialysis, ultrafiltration and biosorption; [36]. At present, phytoremediation (plants or microorganisms) is being used at an early stage to extract heavy metals from water, sediment and soil by concentrating them in their organic matter.

10. Conclusion

Increased regular releases of anthropogenic activities such as untreated poultry waste containing heavy metals into the aquatic environment globally, particularly in underdeveloped countries, could pose a risk to aquatic species as well as affect the ecological balance that can be transmitted via the food chain to humans and could pose serious human health problems. Regulation of the use of heavy metals as an additive or complete elimination of heavy metals in animal feed should be carried out in order to mitigate the human health risks associated with the use of animal products and the contamination of the atmosphere by manure. Preventive steps should be taken to minimize the level of heavy metal contamination in the marine ecosystems by destroying animals when the concentration is too high or consumed and transmitted to humans.
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