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Himalaya Bhardwaj
Department of Veterinary Physiology and Biochemistry, GADVASU, Ludhiana, Punjab, India

Chanchal Singh
Department of Veterinary Physiology and Biochemistry, GADVASU, Ludhiana, Punjab, India

BV Sunil Kumar
College of Animal Biotechnology, GADVASU, Ludhiana, Punjab, India

Digvijay Singh
Department of Veterinary Physiology and Biochemistry, GADVASU, Ludhiana, Punjab, India

Corresponding Author:
Himalaya Bhardwaj
Department of Veterinary Physiology and Biochemistry, GADVASU, Ludhiana, Punjab, India

Role of metallothionein to moderate heavy metals toxicity in animals: A review

Himalaya Bhardwaj, Chanchal Singh, BV Sunil Kumar and Digvijay Singh

Abstract
With the pace at which the population is increasing in India, there is a constant increase in food demand and livestock products. Simultaneously, it increases burden on the industries to produce more. Pollutants from industrial waste, which contain highly toxic heavy metals, enter into food chain reaching to animal body. Toxic heavy metals (lead, arsenic, cadmium) have various side effects on different organs of body like liver, kidney and thyroid. Many studies were conducted in the past to see the levels of heavy metals in blood, soil, milk and organ of buffaloes and cattle. Even though heavy metals are the oldest known toxins harmful to animals, heavy metal toxicity is still a topic that requires further investigation. Metallothionein plays important role in binding of heavy metals like cadmium and mercury by replacing zinc or copper. However, very less is known about metallothionein in buffalo and cattle. In this review we have tried to concise the information available in literature about heavy metals in livestock (tissues and blood) with reference to metallothionein.

Keywords: metallothionein, moderate heavy metals toxicity, animals

Introduction
Agriculture and animal husbandry in India have been intricately woven into each other since the time immemorial. Livestock plays an important role in the economy of the country. Livestock sector contributes 4.11% in GDP and 25.6% of total agricultural GDP. India is first in total buffalo's population in the world. According to 2012 census survey, India has 105.3 million buffalo's population contributing a major portion of milk and its product [1]. Besides milk production, its contribution in fuel and fertilizers production from the farm waste, serving as a source of meat, hides and drought power makes it an integral part of livestock economy of the country [2].
Overpopulation causes increase in food demand and livestock products. To full fill ever-increasing demand of food, dairy product and essential need of population modernization of industry is imminent and causes use of excessive fertilizer in agricultural land due to which increase in environmental concentration of heavy metals in water, feed, air and soil has taken place [3]. Food chain contamination by heavy metals has become a major issue in recent years because of their potential accumulation in biosystems through contaminated water and soil. Due to large-scale production, consumption and lack of regulations, heavy metals are discharged into the environment in large quantities through waste water irrigation, solid waste disposal, sludge application, vehicular exhaust and atmospheric deposition. As a result, heavy metals are omnipresent in the industrial, municipal and urban runoff. Farm animals, especially cattle and buffalo are very useful bio indicators of environmental pollution leading increase of heavy metals in animal body [4]. This review has been planned because very scare study has been present on role of metallothionein in livestock animal which having high heavy metal level in body.

Heavy metals
The term heavy metals have been widely used as a group name for metals and metalloids that have been associated with contamination and potential toxicity. In terms of toxicity, elements commonly used in industry and generically toxic to animals and to aerobic and anaerobic processes, but not everyone is denser or entirely metallic. Includes: As, Cd, Cr, Cu, Pb, Hg, Ni, Se, Zn [5].
Heavy metals like zinc (Zn), copper (Cu), chromium (Cr) arsenic (As), cadmium (Cd) and lead (Pb) are potential bio- accumulative toxins for the dairy production system [6].

Therefore, when ingested in large quantity via food chains are accumulated in the organism, which causes deleterious effect on animal and human health leading to toxicity. Farm animals, especially cattle and buffalo are very useful bio indicators of environmental pollution [7].

Table 1: Sources and toxicological side-effects of some heavy metals

Heavy metals	Sources	Side effects	References
Arsenic	Dipping and spraying of animal to control ecto-parasites	Skin damage, circulatory problem, and increase risk of cancer development	[8, 9]
Lead	Industrial polluted waste, Highway crops	Major organ like Liver, Kidney, Brain Damage.	[10, 11]
Cadmium	Mining, smelting, and manufacturing of batteries, pigments, stabilizers, and alloys	Lungs, Kidney, Bone Damage	[12]
Chromium	Tannery facilities, chromate, ferrochrome and chrome pigment production, stainless steel welding.	Kidney damage	[13]

Heavy metals like zinc (Zn), copper (Cu), chromium (Cr) arsenic (As), cadmium (Cd) and lead (Pb) are potential bioaccumulative toxins for the dairy production system [14]. These are widespread pollutants of great environmental concern as they are non-degradable, toxic and persistent with serious ecological ramifications on aquatic, soil and animal ecology [4, 15-21].

Heavy metals like chromium (Cr) arsenic (As), cadmium (Cd) and lead (Pb) are potential bio accumulative toxins for the dairy production system [14] and may cause deleterious effects on animal. Micro minerals like Zinc, copper, iron etc. are very essential part of animal ration. Their optimum quantity inside the body is essential for survival and proper functioning of the animal. As these are present in trace amount in the nature, their deficiency is usually frequented in animal corresponding to increase in heavy metals level in animal body [4]. Heavy metal exposure causes alteration in the expression of antioxidant enzymes and cellular proteins. Heavy metals cause extensive tissue and cellular damage, which is largely due to imbalance between reactive oxygen species (ROS) production hindering normal function by displacing the essential metal ion from the enzyme biomolecule leading to loss or inhibition of its activity and resulting in physiological and biochemical alterations in animals and defense presented by antioxidants [4, 18, 19, 22].

Lead (Pb) and its toxicity

Lead is the least mobile element among toxic metals, which is attributed to the binding of the metal to organic matter [23]. It is the most common toxic and the most abundant contaminant of the environment with bluish or silvery-grey color [10, 11, 24-26]. Lead being an abundant mineral with worldwide distribution pose threat to animal health and is accumulated in the environment (Table) by industrial pollution [10, 11, 26]. Cattle are poisoned more frequently than sheep and horses [27, 28].

Lead behaves like calcium in the body and accumulates in bone, liver, kidney and other tissues. The problem of lead poisoning in animals has widely been recognized which needs a special attention for the environmentalist and health personnel. It is a cumulative tissue poison and gets stored in different parts of the body especially in bones, liver, kidney and brain. Besides, direct ingestion of lead leading to increased blood lead levels, accumulated lead in the body also acts as a significant source of blood lead burden [27]. Lead produces mainly acute or chronic poisoning. In acute lead poisoning case fatality in lead poisoning may go up to as high as 100%. In acute lead toxicity in cattle, there is sudden onset of signs and the animal at pasture may succumb within 24 hours.

Lead can substitute calcium even in picomolar concentration affecting protein kinase C, which regulates neural excitation and memory storage [29]. In this outbreak, 10 animals showed clinical signs of lead poisoning, 5 dead and others saved after chelating therapy with $CaNa_2EDTA$ [30].

Arsenic and its toxicity

The organoarsenicals in food are one of the most poisoning in livestock now days because of the displacement of arsenic form almost all phases of farming activities. The common of source of arsenic is in fluid used for dipping and spraying of animal to control ecto-parasites. It has a semi metallic property, is prominently toxic and carcinogenic, and is extensively available in the form of oxides or sulfides or as a salt of iron, sodium, calcium, copper, etc. [31]. Arsenic is the twentieth most abundant element on earth and its inorganic forms such as arsenite and arsenate compounds are lethal to the environment and living creatures. Humans may encounter arsenic by natural means, industrial source, or from unintended sources. Drinking water may be contaminated by use of arsenical pesticides, natural mineral deposits or inappropriate disposal of arsenical chemicals [8, 9]. Occupational and community exposure to arsenic from the activities of humans through the smelting industry, the use of gallium arsenide in the microelectronics industry, and the use of arsenic in common products such as wood preservatives, pesticides, herbicides, fungicides, and paints.

The toxicity of arsenic-containing compounds depends on its valence state (zerovalent, trivalent, or pentavalent), its form (inorganic or organic) and factors that modify its absorption and elimination. Inorganic arsenic is generally more toxic than organic arsenic, and trivalent arsenite is more toxic than pentavalent and zero-valent arsenic.

Once absorbed into the body, arsenic undergoes some accumulation in soft tissue organs such as liver, spleen, kidney, and lungs, but the major long-term storage site for arsenic is keratin-rich tissues, such as skin, hair, and nails. Acute arsenic poisoning is infamous for its lethality, which stems from arsenic destruction of the integrity of blood vessels and gastrointestinal tissue and its effect on the heart and brain. Chronic exposure to lower levels of arsenic results in somewhat unusual patterns of skin hyper-pigmentation, pheriphal nerve damage manifesting as numbness, tinghing and weakness on the hands and feet, diabetes and blood vessel damage resulting in a gangrenous condition affecting the extremities [32].

Clinical signs of arsenic toxicity in cattle vary from gastrointestinal to nervous signs. Arsenic were killer including sodium or sodium arsenate, Arsenic pentaoxide and monosodium or disodium acid. It toxicity produces goiter in rats, and inhibited the growth of rumen bacteria in pure culture as well as reduces the fermentative activity. Chronic arsenic toxicity is mostly manifested in weight loss, capricious appetite, conjunctively and mucosal erythematic lesion including mouth ulceration and reduce milk yield [12].

Cadmium

Cadmium is the seventh most toxic heavy metal [12]. It is highly toxic to human and animals [10, 24-26] and not essential to physiological and biochemical functions It is a by-product of zinc production which humans or animals may get exposed to at work or in the environment. Once animal absorbed these metals, it will accumulate inside the body throughout life. Cadmium is a toxic to virtually every system in the animal body [24].

The kidney is the critical organ in human and mammals exposed for long periods to relatively small amounts of cadmium that might occur in foods [33]. Long-term exposure to cadmium leads to several morpho-pathological changes in the kidneys. Cd accumulates in the kidney because of its preferential uptake by receptor-mediated endocytosis of freely filtered and metallothionein bound Cd (Cd-MT) in the renal proximal tubule. Internalized Cd-MT is degraded in endosomes and lysosomes, releasing free Cd_2 Into the cytosol, where it can generate reactive oxygen species (ROS) and activate cell death pathways.

Chromium

Chromium is present in rocks, soil, animals and plants. They can occur in many different states such as divalent, four-valent, five-valent and hexavalent state. Cr (VI) and Cr (III) are the most stable forms and only their relation to human exposure is of high interest [34].

Chromium (III), on the other hand, is an essential nutritional supplement for animals and humans and has an important role in glucose metabolism. The uptake of hexavalent chromium compounds through the airways and digestive tract is faster than that of trivalent chromium compounds [13].

Nickel

Nickel (Ni) is an essential trace element for many species. In animals, nickel deficiency has been associated with depressed growth, alterations in carbohydrate and lipid metabolism, delayed gestation period, fewer offspring, anemia, skin eruptions, reduced haemoglobin and hematocrit values, hematopoiesis and alterations in the content of iron, copper, and zinc in liver and reduced activity of several enzymes like hydrogenases, transaminases and α-amylase [35].

The highest nickel concentrations were found in the kidneys and lungs, whereas nickel concentrations in the liver were low. Several reports indicate that trans-placental transfer of nickel occurs in animals. Metal accumulation was not detected in the blood, brain, hair, small intestine, liver, and testes of adult male rats fed 10 or 20 mg Ni/kg body weight (as NiCI2) for 14 days [36].

Cobalt

Cobalt (Co) is a mineral constituent of cobalamine or vitamin B_{12}. It helps in the formation of red blood cells and also maintains nerve tissue. The effect of cobalt in the rumen is to participate in the production of vitamin B_{12} (Cyanocobalamine). The first clear evidence that it is a dietary essential was in 1935 from Australian research into the cause of certain diseases of cattle and sheep known as "coast disease" and "wasting disease" [37]. Ruminants and horses essentially require Cobalt in the diet, which is converted into vitamin B_{12} by gastrointestinal microbes. Cobalt is required in the diet at the rate of 0.10 ppm, on dry matter basis for ruminants. Cobalt in the form of vitamin B_{12} is interrelated with iron and copper in haematopoiesis and thus, indirectly involved with molybdenum [38].

Copper

Copper (Cu) is an essential trace mineral required for many biological processes [37]. It is involved in the formation of number of Cu containing proteins that have enzymatic functions [39, 40] such as peroxidase, cytochrome oxidase, and superoxide dismutase (SOD). Most Cu in plasma is bound to ceruloplasmin (CP) secreted from the liver, although copper entering blood after intestinal absorption is bound to albumin or amino acids [39]. CP is the reliable indicator of copper status in animals. Copper takes part in the process of osteogenesis, helps in pigmentation and keratinization of hair, essential for erythropoiesis, involved in ceruloplasmin, copper-containing enzyme regulated iron absorption. Sway back in young lambs has been reported due to copper deficiency in livestock [40].

Zinc

Zinc (Zn) is involved in protein synthesis, carbohydrate metabolism and nucleic acid metabolism, through its association with enzyme systems either as a metalloenzyme or as an activator of enzymes [41]. It acts as an important mineral which is an essential component of both DNA and RNA polymerase enzymes. It is a component of a large number of important metalloenzymes *viz*. carbonic anhydrase, carboxy peptidases A and B, alcohol dehydrogenase, glutamic dehydrogenase, D-glyceraldehyde -3 -phosphate dehydrogenase, lactic dehydrogenase, alkaline phosphatase and others [42].

Zinc deficiency interferes with hepatic synthesis of retinol binding protein, resulting in an impaired mobilisation of retinol from the liver [43]. Zinc prevents and cures parakeratosis [44]. Most of the zinc in blood stream (80%) is present in the erythrocytes, which contain about 1 mg Zn per 106 cells, of which 85% is present as carbonic anhydrase and about 5% as Cu-Zn superoxide dismutase [45].

Iron

Iron (Fe) is the most abundant trace element in the body which is a part of many enzyme systems *viz*. hemoglobin, myoglobin and cytochrome. Approximately 60% of body iron is present as haemoglobin, the molecule chiefly involved in oxygen transport. It carries oxygen from the lung via arterial blood as oxyhaemoglobin and it returns as carboxyhaemoglobin carrying CO_2 via venous circulation. Iron being a part of cytochrome enzyme iron participates in electron transport chain. Myoglobin is a simpler ferrous porphyrin found in muscle. About 25% of body iron is in the storage forms, ferritin and hemosiderin, found in liver, spleen, bone marrow and other tissues [46].

Heavy metal in animal's tissue and blood

Heavy metals are predominantly environmentally toxin contaminants. They are distributed widely by deterioration, agricultural and industrial processes through leaching into the surroundings. They also exist naturally in soils at low concentrations. High concentration of heavy metals make them very important group of environmental toxicants since they are potent metabolic poison to humans, animals, fish and plants [47]. The dumping of effluent and wastewater sludge is responsible for heavy metal pollution in rural areas, which is problematic for grazing animals because heavy metal ions are deposited on pastureland, fodder, grasses or forages [48].

Animal tissues, including blood contaminated with heavy metal, are a major source of concern for dietary safety. They are hazardous and may pose a health risk even at extremely low levels [49]. Miranda *et al.* [50] reported high levels of some toxic and trace metals in calves and kids from a polluted area of Northern Spain even as Jukna *et al.* [51] reported moderate levels of heavy metals in the viscera and muscles of Lithuanian cattle. In addition, high levels of certain heavy metals were also confirmed in livestock reared close to a metallurgical industry [52]. In urban areas the blood lead (Pb) in bovine animals was significantly higher than in rural areas. Lead toxicosis was less familiar in buffaloes than cattle, but equally fatal in buffaloes [53]. Relative metal concentrations in the plasma of dairy animals were found to be relatively higher Pb (0.09 ± 0.03 ppm) and Cd (0.065 ± 0.014 ppm) than Cu and Zn (normal) of peenya industrial city in the peri-urban region of Bangalore City. Dey and Dwivedi [54] have examined the equines blood having excessive environmental lead in urban areas. Intake of lead contaminated forages could be the contributing factor for this condition [55].

It is noteworthy that in different corpses, heavy metals tend to build up, but especially in essential organs such as skin, kidneys, blood, stomach and intestines [56] Nigeria. This study has been conducted to measure and entrails of slaughtered animal levels of these toxicants in blood. In the proximity of a metallurgical industry, studied the concentration in cattle of different heavy metals. For the case of excessive metal 'Cd'; 'Pb'; 'Ni'; 'Zn'; 'Cu' and 'Fe' tissues and organs (n = 42) of 21 cattle (muscular, liver) were analyzed. In livers 'Pb' 1.072; 'Cd' 0.456; 'Zn' 79.946; 'Cu' 84.091; 'Fe' 146.822; 'Ni' 0.231 mg/kg, respectively, the highest average levels of heavy metals have been recorded. The highest average levels of the muscle were 'Pb'; 'Cd'; 'Zn'; 'Cu'; 'Fe'; 'Ni' 0.671, and 81.180, 6.312, 51.800, respectively, 0.350 mg/kg. These results concluded that lead and cadmium are of particular ecological importance in cattle or ruminants.

In a study reported by Lopez *et al.* [57], he measured wet wt. concentrations of three toxic elements, arsenic, cadmium, and lead along with two trace elements copper and zinc that were analysed in the liver (Li), kidney (Ki), muscle (M) and blood (Bl) of calves (males and females) between 6 and 10 months old and cows 2-16 years old from Galicia, NW Spain. Sex had almost non-significant effect on the amount of toxic metal accumulated except that kidney cadmium concentrations were significantly higher in females than males. With age, accumulation of cadmium and lead (but not arsenic) concentrations in most tissues were significantly greater in cows than female calves. Female calves had significantly higher levels than males of muscle zinc and blood copper and zinc. Female calves accumulated more copper but less zinc in the liver and kidneys compared with cows; this might have been associated with the chronic, low-level cadmium accumulation observed in cows.

Studies in cattles suggest that females accumulate increased Cd in kidneys compared with males [57]. The form of Cd administered may affect the degree of nephrotoxicity. A single injection of Cd bound to MT at doses as low as 0.2 mg/kg was nephrotoxic in mice, whereas administration of Cd chloride up to 3 mg/kg did not affect renal function [58]. In animals, cadmium effectively induces cancer at multiple sites and by various routes [59]. The concurrent poisoning of lead (Pb) and Cd in sheep and horses near a non-ferrous metal smelter in China. Affected horses mean blood Cd concentrations were higher as compared with control horses blood concentrations. While mean blood Cd concentrations in the affected sheep were very observed high as compared with the control animals [60].

Despite the similar blood concentrations, kidney, liver and muscle concentrations were higher in the calves located in the industrial area compared with those located in a rural area; suggestive that although exposed to higher Cd, this is not always associated with raised blood concentrations. Research conducted in India, report substantially higher concentrations of blood Cd, using similar methodologies. Patra *et al.* [11] determined blood and milk Cd concentrations in 210 lactating cows reared and kept within 2 km radius of a number of different industrial units or in a non-polluted area to serve as controls. Their results are suggestive that cows reared and kept near a steel manufacturing plant had higher blood Cd concentrations (mean 232 μg/l; ranging from 90 to 410 μg/l) compared with cows kept near other industrial sites or in a non-polluted area (mean 28 μg/l; ranging from 0 to 50 μg/l). Interestingly, further research by the same group suggests that whole blood Fe was lower in the cows near the steel processing plant compared with those in the non-polluted areas [10]. Many studies worldwide have reported the concentrations of Cd in meat, liver and kidneys of meat producing animals. More recent work suggested that samples obtained from calves in industrialized areas have higher concentrations of liver and kidney concentrations of Cd [61, 62]. Age was highly associated with kidney Cd concentrations and older cows had much higher concentrations of Cd compared with younger animals.

Sharma and Joshi [63] conducted a survey in Rudraprayag, Pauri, New Tehri, Uttarkashi, Chamoli and Dehradun districts of Garhwal region of Uttaranchal. Fodder, Soil, and serum samples were collected and analysed for their micro mineral content. Significant deficiency of Zn, Co and Cu was observed in soil, fodder and serum samples of these areas. The significantly low soil, Zn, Co and Cu value was observed in Chamoli, Uttarkashi and New Tehri respectively. The minimum serum Zn, Cu and Co was observed in Dehradun (0.64 ± 0.11, 0.34 ± 0.62 and 0.017 ± 0.02 ppm respectively). Kumar *et al.* [64] conducted a study in Agra, Aligarh, Hathras and Mathura districts of Agra region of Uttar Pradesh. The results showed the marked deficiency of zinc; marginal deficiency of copper and cobalt was found in fodder and soil of this region.

Swarup *et al.* [27] estimated the blood and milk lead levels in cows reared around industrial areas. The highest blood and milk lead levels were detected in animals reared in the vicinity of lead-zinc smelter units, followed by aluminum and steel processing plants. Patra *et al.* [10] investigated the trace mineral profile in blood and hair of cattle exposed to lead and cadmium and revealed an increment in lead concentration of blood. The results revealed that increasing blood lead concentration was associated with declining blood copper and iron levels and cows with blood lead level above 0.6 ng per mL had significantly lower blood copper and iron. The blood cadmium level from cattle around lead-zinc smelters was comparable to unpolluted areas. The higher blood lead concentration in cattle affected trace mineral profile in blood and hair. Swarup *et al.* [26] studied heavy metal levels in adult cows reared in polluted environment around different industrial units in India. The study revealed a significant elevation in the blood lead level in cows reared around lead-zinc smelters.

Shahjalal *et al.* [65] investigated the micro mineral profile of feed at four agro-ecological zones (Haluaghat, Nandail, Trishal and Karimgonj upazila) in Mymensingh district. The non-significant differences (P>0.05) of copper (Cu), zinc (Zn), iron (Fe) and manganese (Mn) in paddy straw, road side grass and fallow land grasses for different land type were observed. The average mineral concentration (ppm) of straw was 6.12, 24.93, 291.3, and 224.9 for Cu, Zn, Fe and Mn respectively. The higher values for Fe and Mn contents and lower contents of Cu and Zn contents were observed. There were plenty of "Cu"; "Zn"; "Fe" and "Mn" both on the road side and on the pasture ground. Blood serum "Cu" and "Zn" content (P>0.05) varied not substantially between animals in four types of land. Based on 0.65 ppm serum Cu as critical level indicated a higher percentage of samples was below the critical level. Serum Fe and Mn concentrations of all samples were above the critical level of 1.0 ppm and 20µg/ml respectively and the animals were not deficient in Fe and Mn.

Sudhaharan [66] observed the maximum mean serum copper value (0.61 ppm) in dairy cow reared in Karur district than rest of the districts in Tamil Nadu. Dzoma *et al.* [67] studied the influence of environmental levels on the heavy metal levels in serum and faeces. The occurrence of trace metals in grass and livestock specimens indicated the upward mobility of heavy metal pollutants through the food chain.

Roy *et al.* [68] experimented heavy metal contents in milk samples of cow and buffalo from Haryana. Lead (Pb) content of cow and buffalo milk showed significant differences with mean values of 0.035 ± 0.009, 0.048 ± 0.009 and 0.100 ± 0.007, 0.090 ± 0.013 ppm in industrial and non-industrial areas respectively. Significantly higher Pb values were observed in cow (0.091 vs. 0.065 ppm) as well as buffalo milk (0.107 vs. 0.059 ppm) samples collected from industrial locations. Cadmium (Cd) content of cow milk was significantly (P<0.05) higher in non-industrial as compared to industrial area (0.008 vs 0.004 ppm). Cd content in milk of two species from industrial and non-industrial areas was comparable. Mercury (Hg) content in buffalo milk varied from 0 to 2.78 ppb and 0 to 1.3 ppb in the two zones. Buffalo milk from industrial area contained significantly (P<0.05) higher Hg than non-industrial area.

A study reported by Malik *et al.* [69] on eight female genitalia of Murrah buffaloes and results revealed that the ovaries contained the highest concentration of iron. Zinc and manganese were also higher in the oviduct. The cervix had higher levels of iron and copper as compared to the vaginal tissue. Uterine and vaginal tissues exhibited the lower concentrations of iron, copper, zinc and manganese.

Akhtar *et al.* [70] planned a study during the months of march to june to determine serum micro-mineral status (copper, iron, zinc, selenium) in cyclic and anoestrus buffaloes. Mean serum concentrations of copper, iron, zinc and selenium in cyclic buffaloes were 70.59µg/dl, 370µg/dl, 181.4µg/dl and 0.10µg/ml, respectively. The corresponding values for anoestrus animals were 62.23µg/dl, 358.13µg/dl, 164.07µg/dl and 0.07µg/ml. Compared with cyclic buffaloes, there were significantly lower serum copper, iron, zinc and selenium levels in anoestrus buffaloes (P<0.05). It was concluded that deficiencies of copper, iron, zinc and selenium either singly or in combination, might be responsible for anoestrus condition in Nili-Ravi buffaloes.

Tomza-Marciniak *et al.* [71] estimated the serum concentration of cadmium, lead, iron, zinc, copper, chromium, nickel, aluminum and arsenic in cattle from organic and conventional farms. The results revealed significantly lower serum concentration of heavy metals except cadmium in animals of organic farm than conventional farm. The serum lead concentration was significantly correlated with cadmium, zinc, iron, copper and nickel. The study demonstrated zinc deficiency in 14 and 95 percent cows from conventional and organic farm, respectively.

Bhat *et al.* (2011) collected forage samples from Ganderbal district of Kashmir and analysed them for different macro and micro mineral content and found that all the mineral values were above critical level (Copper = 10 ppm, Iron = 30 ppm and Manganese = 40 ppm) except zinc (Zinc = 30 ppm) and in serum of the cattle highest deficiency was observed for copper (50%) followed by phosphorus and calcium (37.5%).

Mudgal *et al.* [73] conducted an experiment to determine serum micro-mineral status (iron, copper, zinc, manganese, cobalt) in 50 anoestrus Murrah buffalo, Sahiwal and Crossbred cows and heifers. The level of copper was on a border way to show the signs of clinical deficiency symptoms, while the levels of other heavy metal elements was in quite safe range to encounter any problem. It was thus concluded that deficiency of copper, could be responsible for anoestrus condition in these animals.

Devi *et al.* [74] conducted a baseline survey in different blocks of Idukki and Ernakulam districts of Kerala state with a view to access the major micro-minerals i.e., copper (Cu), Zinc (Zn) and Iron (Fe) status of plant/fodder in two districts of Kerala and observed that Cu, Zn and Fe contents of fodder were 9.29 ± 0.21µg/gm, 41.16 ± 1.8µg/gm and 710.70 ± 14.48µg/gm with deficiency of 38.66%, 38% and 0%, respectively. 160 serum samples included in survey and overall prevalence of Copper, Zinc and Iron deficiency in serum samples of cattle were 46.87%, 40.00% and 15%, respectively in the two representative districts of Kerala. Chhabra *et al.* [75] conducted a survey on mineral (Ca, Pi, Zn, Cu, Fe, Mn) status of dairy animals and fodder in a total of 67 dairy units of 29 villages of central Punjab during months of June in summer and January in winter. A total of 188 buffaloes were selected randomly without considering any health problem or mineral deficiency symptoms and reported that the plasma samples contained higher levels (P<0.05) of Zn and Pi during winter as compared to summer. Forage had statistically non-significant (P>0.05) levels of Calcium, Iron, Cu and Mn during both seasons; contents of the Ca, Fe, Cu were higher during winter than those during summer though the difference was not statistically significant.

Panda *et al.* [76] planned a survey based study in North Eastern Ghat (NEG) of Odisha to analyse the mineral profile of soil, feed, fodder and serum of dairy cattle. Cu and Fe content of feed and fodders were marginally higher than the critical level whereas phosphorus was found severely deficient. The Ca content of the soil in the surveyed zone was 0.12% which is much below the critical level (0.80%). The phosphorus status of the soil of NEG is below the critical level. This can be attributed to the low pH of the soil in NEG region and the ionic acidity of the soil induces the formation of complex with Fe and the availability of inorganic phosphorus is reduced. Higher Fe status of this region showed that the soil is very rich in Fe. This is because most of the Fe and Mn mines are located in Odisha. Cu content of soil, feed and fodders of this zone is marginally higher than the critical level, the deficiencies of Cu is observed in the serum of cows which might be due to poor bioavailability of Cu resulted from the increased lignification of the fodders in tropical countries [77]. Serum Fe concentration in most of the animals in surveyed zone were above critical level even without any mineral supplementation. This might be due to abundance of Fe in feed and fodder grown of naturally Fe rich soil.

Devhane [78] conducted a survey on 24 Pandharpuri female buffaloes maintained at College farm of KNP College of Veterinary Science, Shirwal. The Pandharpuri buffaloes were categorized into different groups according to age comprising 6 (six) animals in each group *viz*. Group I: Buffalo calves (up to 6 months), Group II: Young growing buffalo calves (6 months to 12 months), Group III: The present data reveals that, there was non-significant increasing trend in concentration of serum copper among different age groups with the values in the range from 1.92 ± 0.30 ppm to 2.46 ± 0.31 ppm. There was a significant apparently increasing trend in concentration of serum iron among different age groups with the values in the range from 1.14 ± 0.15 ppm to 2.45 ± 0.15 ppm. There was no significant difference in concentration of serum zinc among different age groups with no consistent trend. The values in these groups range from 1.15 ± 0.15 ppm to 1.43 ± 0.14 ppm.

Sarma *et al.* [79] carried out a study of mineral status of dairy cows in relation to commonly fed tree fodders, soil and serum mineral status were studied in Mizoram and it was found that Cu, Zn and Fe were optimum in all studied locations. Except Ca, other minerals were estimated to be optimum in the tree fodders. The soil Ca, P and Mg were observed below the critical levels; but Cu, Zn and Fe were optimum in all studied locations and in serum found that all minerals were found to be deficient except Fe in the serum of dairy cattle.

Bhagat *et al.* (2017) studied the mineral profile in relation to feed and fodder, soil and blood serum of dairy animals was carried out in selected blocks of Sindhudurg district of Maharashtra to identify the macro and micro minerals profile. Feeds and fodder samples were also deficient in Ca, P, Mg and Zn content. Low serum Ca, P and Mg was probably due to their low content in locally available feedstuffs and Cu, Zn and Fe content of blood serum of dairy animals reared on non-irrigated and irrigated condition was 1.83 and 1.67 $mgdl^{-1}$, 0.89 and 0.97 $mgdl^{-1}$, 3.18 and 2.87 $mgdl^{-1}$, and 1.14 and 1.15 $mgdl^{-1}$, respectively which is above the critical concentration. The findings indicated the adequate Cu level in serum and content of Ca, P and Mg was found deficient in blood serum in dairy animals. The average Ca, Mg and P were deficient in blood serum, whereas Cu, Zn and Fe were adequate in blood serum of dairy animals.

Heavy metal exposure in dairy animals (In Punjab, India)

As, Cd, Cr, Cu, Mn, Ni, Pb and Zn were detected in the water samples of naturally occurring ponds of villages Killa Raipur and Lohatbaddi of district Ludhiana. The presence of heavy metals in pond water was probably due to the pollution by agriculture runoff, dumping of solid waste and soil erosion [81]. The concentrations of Pb, Cd, Ni and As in the blood of cattle inhabiting Buddha Nallah area of Ludhiana district in Punjab were above the minimum permissible limits; and higher levels of Pb exposure through drinking seepage water were observed in jersey cows within 4 weeks of exposure [7]. The soil, fodder, drinking water and blood of buffaloes were having higher concentrations of arsenic in the arsenic contaminated area of Ludhiana district, Punjab which in turn affected the liver and muscle function [17]. A study to explore the quantity of heavy metals and trace minerals in the groundwater of villages near Buddha Nullah in Ludhiana revealed the concentration of Cr, Mn, Cu, Zn, As, Sb, Cd, Hg, and U was higher than the permissible limits and also found that water is not safe for drinking due to many heavy metals in high concentrations [82]. A study of heavy metals/trace element contamination of groundwater resources in Ludhiana and Patiala in Punjab revealed the heavy metals: Cd, Cr, Pb and Hg above the minimum permissible limits. The textile effluents in and around Ludhiana are highly toxic not only for human beings living near affected areas but also a serious threat to ground and surface water resources [3]. Surface water of Ludhiana is highly contaminated by the effluents released by the industries and sewage. The seasonal pattern for all the heavy metals was same i.e. higher values during summer and lower values during the winter season [83].

The total number of villages affected by heavy metal contamination is 2420 in India, out of which 2139 fall in Punjab, 273 in Bengal, 7 in Assam and only one in Karnataka. An alarming 80% of surface water of India is polluted assessed by an International. Punjab accounts for 88 percent of the total habitations in the country that are adversely affected by the presence of Al, Ni, Cd, Cr, Pb and Hg in groundwater while making up less than 2 percent of India's landmass. Groundwater in 3, 067 of a total of 13, 000 villages in Punjab has been found unfit for drinking due to heavy metal content. As per the report of the water supply and sanitation department, the heavy metals (traces of arsenic) found as deep as 1, 000 feet in these villages. The number of villages affected by these elements in Punjab is much more than that in Haryana and Himachal. As many as 2, 139 habitations or villages in Punjab state were reported to the integrated management information system of the Ministry of Drinking Water and Sanitation as having more than the permissible levels of heavy metals [84].

The heavy metals distributed in groundwater of eight districts in Malwa region of Punjab showed that maximum percent of As, Pb, Fe, Co, Cr, Zn and Hg water samples is beyond the permissible limits compared with standard values given by BIS and not fit for drinking purposes [85]. The increase in the concentration of heavy metals was recorded during summer as compared to winter probably due to drought and decrease in water level, as observed in a comprehensive assessment made on seasonal variations of heavy metals in sirhind Canal along its course in Moga, Punjab in different seasons. A considerable variation was observed in the concentrations of As, Cr, Cu, Fe, Mn, Ni, Pb and Zn at all the ten sampling sites in their study [83]. In central and sub-mountainous Punjab, approximately 32% of tube well water is contaminated by As. The levels of As in the tube well water ranged from 0.42 to 20.97µg/L which were not fit for consumption as per WHO guidelines [86]. The distribution of As in surface soils in southwestern district of Punjab indicated about 39.5% of water samples with arsenic content more than the permissible limit of 10 ppm [87]. In the deep aquifers of central and sub-mountainous districts of Punjab, the presence of As containing minerals may lead to higher concentrations of As in the tubewell waters [88].

Due to haphazard industrial development, the heavy metals entering milk through contaminated food and water that was ingested by the dairy animals which leads to health implications in humans [89]. Animals grazing on the heavy metal contaminated plants and drinking heavy metal polluted waters accumulate such metals in their tissue and milk [90]. Milk samples collected from different districts of Haryana contained Pb, Cd, As and Hg contents below the maximum contamination level and were, therefore, safe for human consumption. Buffalo milk from the industrial area contained significantly ($P<0.05$) higher Hg than non-industrial area; and As and Hg contents were higher in cow milk than in buffalo milk [68]. The sources of contamination of heavy metals Cd, Cu, Ni, Pb and Zn can originate from natural, mining activities, smelting operations, agriculture and enter the animal body through feeds [91].

Metallothionein and its role in animals

Heavy metal exposure in animals causes alteration in the expression of antioxidant enzymes and cellular proteins [4, 19-21]. Several metal binding proteins like metallothioneins (MTs) also play important role in transporting and regulation of heavy metals. MTs was first isolated from horse kidney [92]. They are low molecular weight cysteine-rich intracellular proteins which have high affinity towards d-10 electron group metals which includes essential (Zn and Cu) and non-essential (Cd and Hg) trace elements [93].

MTs are ubiquitous metal binding proteins and have been isolated in a wide variety of organisms from bacteria to humans playing a key role in metal metabolism. MTs have four isoforms MT-1 MT-2 MT-3 AND MT-4. MT-1. MT-2 is expressed in many tissues, particularly kidney, liver, pancreas and intestine whereas MT-3 and 4 are found principally in brain and skin [94-96]. These proteins bind tightly to heavy metals to decrease toxicity [97, 98]. For mammals, MTs bind zinc [99], but with excess copper or cadmium, zinc can be easily replaced by these metals [100]. Because of their rich thiol content, MTs bind a number of trace metals including cadmium, mercury, platinum and silver, and also protect cells and tissues against heavy metal toxicity.

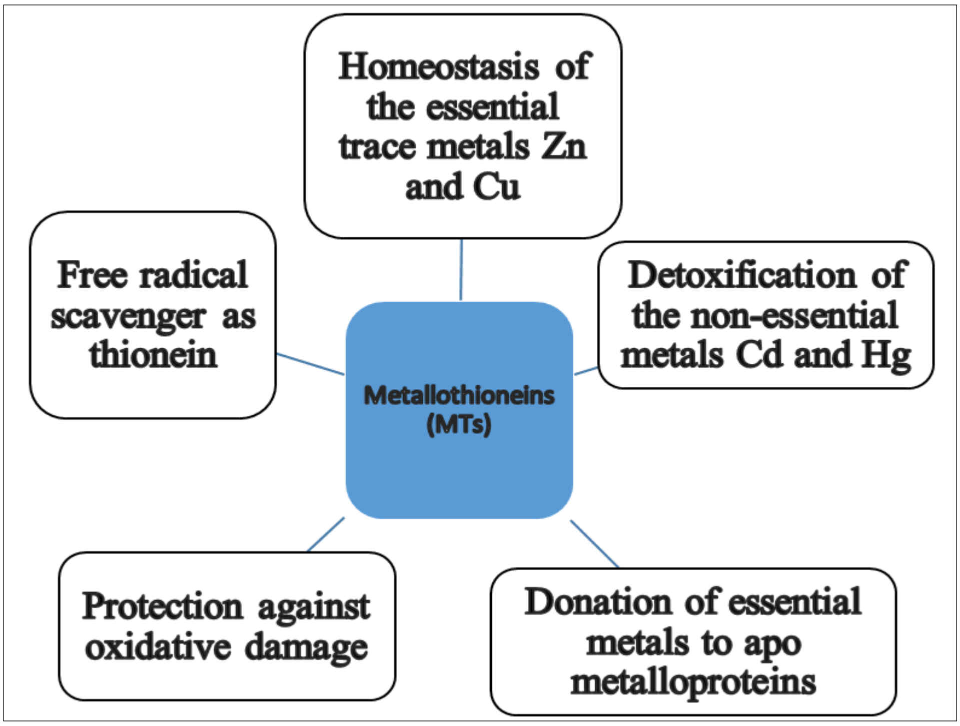

Fig 1: Functions of metallothioneins (MTs) (Adapted from Yang *et al*.; Ruttkay-Nedecky *et al*.) [101, 102]

Apart from antioxidants, metal binding proteins like Metallothioneins (MTs) also regulate the transport of the heavy metals in circulation and tissues. MTs play important role in maintaining the homeostasis of cell by the synthesis of zinc and copper metallo enzymes which bind tightly to heavy metals to decrease toxicity [97].

In a study to investigate metal accumulation and detoxification processes in cattle from polluted and unpolluted areas, Roggeman *et al.* [103] reported that cadmium and lead were significantly higher in tissues of cattle from polluted sites and cadmium seemed to be the most important metal that induced metallothionein (MT) in kidneys. Metallothionein functions in Cd detoxification primarily through high affinity binding of Cd to MT and in the kidneys and liver MT concentrations are high [98]. The rate of excretion of Cd is slower than that of uptake; hence the need to detoxify and store Cd by an immobilization mechanism is a consequence of this slower rate of elimination [104, 105]. Along with glucocorticoids, the essential metals Zn [106, 107] and Cu, and the toxic metal mercury, intracellular Cd induces metallothionein synthesis in many organs including the liver and kidneys. Metallothionein expression however was not affected by Fe status in piglets [108]. Induction of metallothionein synthesis by Zn [106, 107] ensures sufficient MT to bind and detoxify ingested Cd. *In vitro* studies in rats show that intestinal Zn-MT incubated with Cd chelated with cysteine (Cd-Cys), the Cd dissociates from the cysteine and exchanges with the Zn bound the MT, thus allowing the MT to act as a detoxifier and transporter. Mice resistant to Cd toxicity. Thus, MT is critical for protecting human and animal health from Cd.

MTs protect cells from exposure to oxidants and free radicals and play important role in regulation of intracellular Zinc level [109]. It has been found that the expression of MTs has been influenced by metal transcription factor 1 (MTF-1) and several metal ions such as Zn and Cu [110]. MT transcription has been found to be regulated by MTF-1 along with other metal transport proteins in order to respond to other heavy metal ions or oxidative stress. A recent study on variation of DNA sequence of MT of ZEBU cattle has revealed that this MTs could be used as biomarker in heavy metal homeostasis [111].

DNA poly-morphisms in a number of environmentally responsive genes can explain variations in toxic metal biomarker values and health outcomes. Studies on mammals (wildlife, humans and rodents) show toxic metal exposures to be related to epigenetic marks such as DNA methylation [112]. Genetic variation leading to differences in expression and regulation of metallothionein proteins may contribute to observed differences among animals in terms of toxic metal uptake and metabolism.

One of the recent study revealed that the mutations in the partial coding region of the metallothionein gene of Zebu cattle using PCR-RFLP and DNA sequencing molecular techniques. DNA sequence analysis using various bioinformatics tools revealed abundant nucleotide substitutions in MT-2 nucleotide sequences representing toxic metal exposed and unexposed animals. Due to the functional relevance of the metallothionein gene with toxic metal homeostasis, metallothionein is one of the important candidate genes to study in a context with toxic metal homeostasis. Identification of genes for tolerance or susceptibility of toxic metals will be of immense importance for breeding and propagating superior genes. These findings may help explore metallothionein as one of the best DNA markers for toxic metal homeostasis in animals continuously exposed to toxic metals [111].

Recently it has been found that abattoir buffaloes, which was exposed to heavy metal, had increased metallothionein expression in various organs like liver, kidney and blood as compared to non-exposed group [4, 19-21]. When compared between arsenic and chromium exposed group it has been observed that liver has maximum metallothionein compared to orther organs [4, 19-21].

It has been also observed that supplementation of Vitamin E and Se has no effect on heavy metal level in environmentally exposed buffaloes but level of metallothionein found to be increased [20]. Very less research are available on metallothionein role in domestic animals so gives us an opportunity to explore more.

Conclusion

Higher levels of heavy metals in animal organs are a serious concern in India and other countries. The overall situation of increasing heavy metals in animal body are alarming as increased levels of these heavy metals are found in animal organs due to food chain and accumulation may cause severe human health hazards. Metallothionein plays an important role in binding of these heavy metals and sequestering them. However, there is still a dearth of research focusing on the role of metallothionein in bovines.

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