ANIMALS AS BIOLOGICAL INDICATORS FOR HEAVY METAL POLLUTION:

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خلاصہ

بھاری دھاتوں سے ہونے والی آلودگی بڑھ رہی ہے اور ہماری زندگی اور ماحول کو خطرناک حد تک متاثر کر رہی ہے۔ بظاہر معمولی لگنے والی ان دھاتوں کی مقدار حقیقتاً ہمارے گرد موجود فلورا اور فانا کی صحت پر دوررس سنگین نتائج مرتب کر سکتی ہیں۔ جانوروں کو ماحول میں بھاری دھاتوں کی مقدار کے تعین کے لیے بہترین بائیومانیٹر کے طور پر استعمال کر کے آلودگی کو مقداری اور نیم مقداری طریقے سے جانچا جا سکتا ھے۔ لہٰذا جانور دنیا بھر میں ماحولیاتی آلودگی اور خطرناک پلیوٹنٹ کی مقدار کو جاننے کے لیے ایک اہم بائیواِنڈیکیٹر کے طور پر توجہ کا مرکز بن رہے ہیں۔ وقت کے تقاضوں کے تحت پاکستانی سائنسی تنظیم کی جانب سے جانوروں پر کی جانے والی سینٹینل تحقیق کی اہمیت کو اجاگر کرنے کی مسلسل کاوش درکار ہے۔ یہ محققین کی متعلقہ دھاتوں سے ہونے والے نقصانات کی نشاندہی نہ صرف جانوروں بلکہ انسانوں اور ماحول پر بھی کرے گی۔

Abstract

Contamination by heavy metals is threatening our environment and lives. A seemingly insignificant concentration of metals, can in turn be a potential threat to the well being of flora and fauna in the long run. Animals are a good tool to biomonitor the concentration of heavy metals in the environment and therefore pollution levels can be traced quantitatively and semi quantitatively through them. Thus, animals are gaining worldwide attention of their use as bioindicator species to trace environmental contamination by these perilious pollutants. Interminable attempts from the scientific community of Pakistan should be considered to signify the importance of animal sentinel studies. It will help researchers chalk out the risks associated with heavy metals, not only on animals, but also on humans and environment.
Keywords: Heavy metals, Environment, Contamination, Animals, Bioindicators

Introduction

The term "heavy metals" is defined as metals having a density greater than 5 g/cm^3 and are even harmful or virulent in their small amount (Agarwal, 2009). Copper (Cu) cadmium (Cd), chromium (Cr), silver (Ag) lead (Pb), zinc (Zn), iron (Fe), mercury (Hg), arsenic (As), and the platinum group elements comes in the category of heavy metals (Duruibe *et al*., 2007). Microelements play an important role in living beings as they are required by organisms in minute amount for the normal functioning of body. Physiological disorders takes place in an organism either due to the presence in excess or deficiency of certain elements, especially heavy metals, in an individual. Copper, zinc, cobalt, nickel, iron and manganese are important micronutrients since they are required for the growth and development of animals and humans, whereas lead and cadmium have no nutritional value and are considered toxic in nature (Rashed and Soltan, 2005). Therefore, monitoring of contamination caused by heavy metals is of immense importance now-a-days (Merain, 1991).

'Environment' includes everything that surrounds an organism or a group of organisms such as plants, animals, abiotic and biotic factors. Living beings interact continuously with the physical conditions prevailing in their environment which have an impact on the growth of organisms and also influences their ability to survive (Farlex, 2005). A pollutant is defined as any substance in the environment which spoils its well-being, impairs the quality of life by showing objectionable impacts and in grave situation may lead to death. Presence of such toxic substances in the environment must be within their set permissible limit in order to avoid health risks associated with them. Hence, environmental pollution is defined as presence of a contaminant in the environment; water, soil and air at levels which is likely to pose harmful health impacts on living organisms surviving in that contaminated environment (Duruibe *et al*., 2007).

The capacity of environment to nuture life and sustain its inherent values has been largely compromised due to contamination of environment with heavy metals. Although heavy metals are naturally occuring compounds but their dispersion in various compartments of the environment has been triggered mainly by anthropogenic activities. Once heavy metals are released in the environment, they tend to bioaccumulate in the food chain because of their persistant nature. This, in turn, threatens the health of animals, humans and plants and reduces the ability of environment to foster life (Masindi and Muedi, 2018).

Assessment of heavy metal pollution in the environment by examining water, air and soil is quite strenuous and exorbitant as it may require multiple samples to be taken with time in order to avoid discrepancy in metal concentration. In such case, use of bioindicator species to reveal the health status of environment appears charismatic, allowing economical and comparatively doable analysis (Phillips, 1977). Biological indicator species are living organisms such as birds, animals, plants, lichens and microorganisms, which are utilized to determine the contamination status of a particular area. Such organisms play an important role for monitoring the health of natural ecosystem and changing biogeographic patterns prevalent in the environment (Parmar *et al*., 2016). Investigating bioaccumulation patterns of heavy metals in animals can provide adequate information to adjunct the data acquired by the chemical examination of contaminants in inert samples (Ma, 1987). Present review article shows the use of animals being used as bioindicators in order to assess heavy metal contamination in the environment.

Sources of Heavy Metals:

Natural and anthropogenic activities are causing contamination of the the environment and its resources by discharging heavy metals more than it can handle (Herawati *et al*., 2000; He *et al*., 2005). Volcanic eruptions, sea-salt sprays, weathering of rocks, biogenic sources, forest fires and wind-borne soil particles are the natural sources of heavy metal emissions (Masindi and Muedi, 2018). To a large extent, these heavy metals are introduced and dispersed in environment by organic wastes, power generation, refuse burning, transport and industrial effluents. Depending upon their state, i.e gaseous or particulate, heavy metals can be carried by wind from their source to far off places. Heavy metals are washed away from air by rain ultimately becoming a part of land and suface water (Agarwal, 2009). Table 1 provides an overview of different sources which are responsible for the dispersion of heavy metals in environment.

Exposure of Animals to Heavy Metals:

Briskly expanding industrial areas are the major contributors of heavy metal emissions in air leading to its contamination while leaded gasoline and paints, disposal of high metal wastes, coal combustion residues, spillage of petrochemicals, mine tailings, land application of pesticides and fertilizers, animal manures, sewage sludge, wastewater irrigation, and atmospheric deposition are responsible for contaminating soil with heavy metals (Khan *et al*., 2008; Zhang *et al*., 2010). Water contamination by heavy metals occur via industrial diacharges, soil erosion, stormwater runoff and a number of other anthropogenic activities (Pandey and Madhuri, 2014). Soil, water and air pollution by heavy metals may pose at risk the health of animals through: the food chain (soil-plant-animal), drinking contaminated water,direct ingestion or contact with polluted soil, breathing in contaminated air and ingestion of contaminated food (McLaughlin *et al*., 2000; Ling *et al*., 2008) resulting in the accumulation of heavy metals in different organs and tissues of such exposed animals (Horsfall Jr *et al*., 1999; Peplow, 1999). Figure 1 represents the potential exposure pathways of heavy metals to become concentrated in animals.

Use of Animals as a Bio-monitoring tool:

Quality of environment can be accurately reflected by using animals which are sentinels of great significance for toxicological risk assessment in general (Pandey and Madhuri, 2014). Teeth of *Myodes glareolus* (Bank vole) was used as an unveiling clue to judge environmental contamination in a study conducted in Poland. This study suggested that heavy metal exposure to animals and humans can be best ascertained by using teeth as bio-indicators of environmental pollution (Gdula-Argasińska *et al*., 2004).

A research study was conducted in Netherlands to determine heavy metals (Cadmium, copper and zinc) in *Aporrectodea caliginosa* and *Lumbricus rubellus* (earthworms). High level of cadmium and copper was detected in earthworms residing in floodplain soil, suggesting that uptake takes place from the soil contaminated by heavy metals (Hobbelen *et al*., 2006).

Concentration of heavy metals was assessed in the liver of *Cervus elaphus* (red deer) living near a mining area in a research investigation conducted in Spain. Highest concentrations of lead, cadmium, selenium and arsenic was found in the liver of red deer. This study suggested that mining activities were responsible for the accumulation of heavy metals in deer and highlighted the importance of animals as they reflect the health status of their surroundings (Reglero *et al*., 2008).

Ordure of mammals confined in zoo was evaluated and used as a bioindicator of heavy metal pollution in a research work conducted in zoo of Bikaner, India. Cadmium, chromium, copper and zinc were detected in the excrement of experimental mammals. Feed and water given to such mammals in zoo along with the soil in their cages were also analyzed to locate the source of heavy metals. Rigorous investigation specified that due to high traffic density in the area, air pollution was the supreme cause of exposing animals to heavy metal contamination (Gupta, 2013).

A research investigation was carried out on *Capra aegagrus hircus* (goats) nurtured in the region of an industrial area of West Bengal, India. Faeces, tissues of spleen, lung, kidney, liver and muscle were selected for

investigating the concentrations of four heavy metals i.e cadmium (Cd), lead (Pb), cobalt (Co) and copper (Cu). Results demonstrated that these heavy metals were present in greater amount in all the tissues and faeces. Liver contained hightest concentrations of cobalt and copper followed by kidney, lung, spleen, and muscle. However, accumulation trend of lead and cadmium in the tissues of goat were in the following order: kidney> liver > lung > spleen > muscle. Older animals showed greater tendency of accumulating heavy metals in body than young ones. This study suggested that metal accumulation in various organs of goats were due to the contamination of surrounding environment with heavy metals (Kar *et al*., 2015). Table 2 shows previous research studies done on animals to trace metal contamination.

Table 1. Sources of Heavy Metals in the Environment

Sources of Heavy Metals	Phenomena	References
Natural sources	• Volcanoes • Forest and prairie fires • Wind dust which arises from desert region such as Sahara • Igneous rocks such as olivine, augite and hornblende • Sedimentary rocks such as shale, limestone and sandstone	
Agricultural sources	• Sewage sludge • Nitrate fertilizers • Pesticides • Liming	Ross (1994) Seaward and Richardson (1989) Sharma and Agrawal (2005)
Industrial sources	• Petroleum combustion • Nuclear power stations • Coal burning power plants • Processing of plastics • Paper processing • Textile industry • Steel industry • Landfills • Transportation Refuse incineration	

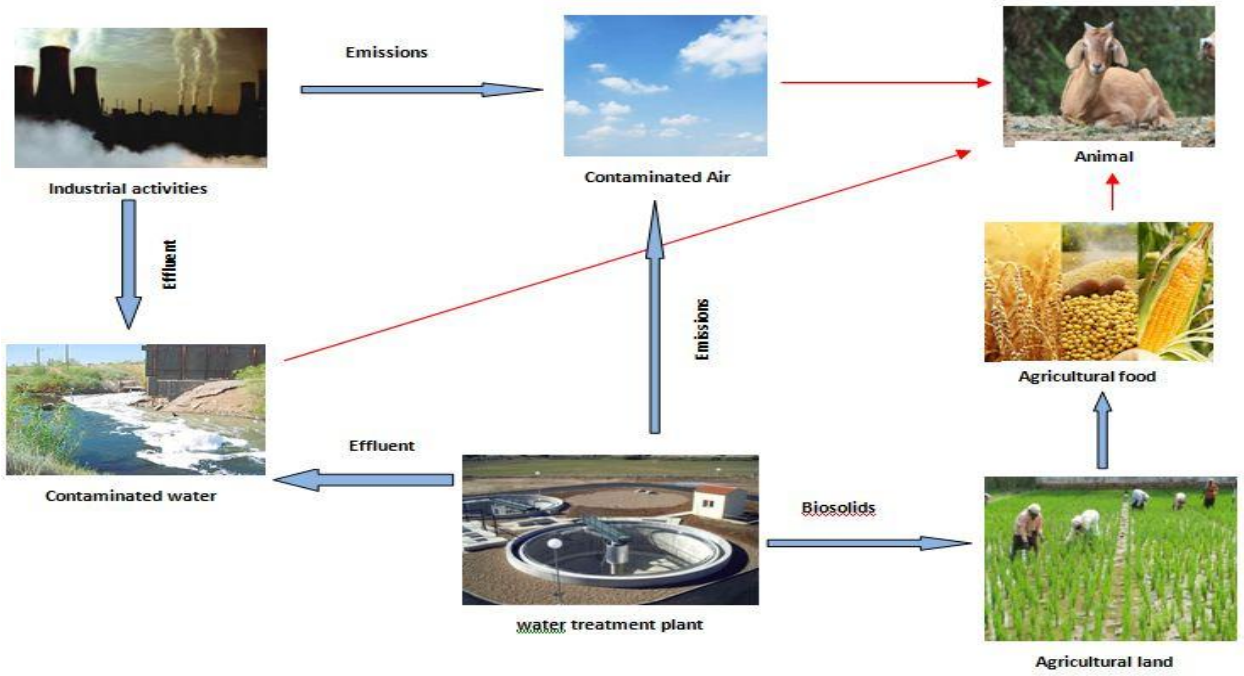

Fig.1. Potential pathways for Heavy Metals to become concentrated in Animals

Health Impacts of Heavy Metals on Animals:

Arsenic, cadmium, lead and mercury are the most noxious heavy metals and are toxic to animals and the environment. Concentrations of heavy metals beyond their set tolerable limit are ruinous. They disrupt the structure and function of natural ecosystems because of their tendency to bioaccumulate in living beings, and are responsible for showing pernicious impacts on biota and even death in most of the living organisms. All heavy metals, despite some of them are essential for human health, have harmful impacts on living beings through genetic mutation and metabolic interference. Heavy metals can bioaccumulate in the body of an organism and food chain (Pandey and Madhuri, 2014). 'Bioaccumulation' means an elevated concentartion of a toxic element in an individual over time, compared to the level of toxicant in the environment (Gupta, 2013). So, in general chronic toxicity is exhibited by the harmful heavy metals (Pandey and Madhuri, 2014).

It is crucial to mention here that the majority of the zoos which were once situated on the margins of towns and cities are now circumscribed by anthropogenic activities, such as industries and road traffic which are causing damage to the environment and its biota on a global scale. All these human activities are destabilizing the environment by polluting it with heavy metals, which in turn may adversely affect the welfare and health of the wild animals harboured in such conservation areas (Gupta, 2013). Heavy metals are carcinogenic, mutagenic and teratogenic in nature and are immuno-suppressive compounds. Chronic exposure to them results in poor body condition and hinderance in the process of reproduction (Pandey and Madhuri, 2014). Table 3 provides an overview of harmful health impacts associated with specific heavy metals in animals.

Table 2. Previous research studies done on Animals to assess Environmental pollution

Detected Heavy metals	Animal used	Selected body part	Area	Reference
Lead, Cadmium, Zinc, Iron	Yellow-necked mice	Liver, Kidney, Testis	Poland	Damek-Poprawa and Sawicka-Kapusta (2003)
Lead, Cadmium, Copper, Zinc	Sheep, Horses	Blood, Hair, Tissues	China	Liu (2003)
Manganese, Iron, Lead, Cadmium, Nickel	Sheep, Camel, Goat	Hair	Egypt	Rashed and Soltan (2005)
Cadmium, Lead, Copper, Zinc	Red deer	Kidney, Liver, Muscle tissues	Poland	Falandysz *et al*., (2005)
Lead	Sheep	Liver, Kidney	Jordan	Massadeh *et al*., (2006)
Mercury	Polar Bears	Liver, Kidney	East Greenland	Sonne *et al*., (2007)
Chromium, Copper, Mercury, Lead, Cadmium, Vanadium, Manganese, Cobalt, Rubidium, Zinc, Strontium, Molybdenum,Silver, Tin, Antimony, Caesium, Barium, Titanium, Bismuth, Indium	Polar Bears	Liver	Northern and Western Alaska	Kannan *et al*., (2007)
Lead, Zinc, Cadmium, Copper, Arsenic, Selenium	Red deer	Liver	Spain	Reglero *et al*., (2008)
Chromium, Nickel, Copper, Lead, Iron	Insectivore Bats	Liver	Brazil	Zocche *et al*., (2010)
Cadmium, Zinc, Lead	Bats	Kidney, Liver, Pectoral muscles	Czech Republic	Pikula *et al*., (2010)
Chromium, Copper, Nickel, Lead, Zinc	Silver and Red foxes	Hair,Skin	Poland	Filistowicz *et al*., (2011)
Lead, Cadmiun, Copper, Cobalt	Goat	Liver, Kidney, Lung, Spleen, Muscle	India	Kar *et al*., (2015)

Table 3. Harmful health impacts of Heavy Metals on Animals

Heavy Metals	Health impacts	Reference
Lead	• Damage to nervous system • Kidney damage • Disruption in reproductive system • Joint problems	Pandey and Madhuri (2014)
Arsenic	• Irritation of lung, intestine and stomach • Brain damage	
Cadmium	• Bone defects • Kidney dysfunction • Myocardial diseases • Increased blood pressure	
Mercury	• Damage to brain • Damage to chromosomes • Kideny damage • Neurotoxicological disorders • Tremors • Damage to central nervous system (CNS)	
Chromium	• Damage to nerve tissues • Liver damage • Kidney damage	
Nickel	• Decrease in body weight • Liver damage • Heart damage	
Antimony	• Nausea • Vomiting • Diarrhoea	

Previous Research Studies on Toxicity of Heavy Metals in Animals:

Technological and industrial advancements are responsible for the rapid dispersion of toxic heavy metals in the environment now-a-days. Liver and kidney damage are the results of cadmium exposure in rats. Cadmium once enters the body of animals, involves oxidative reactions such as cadmium-induced lipid peroxidation (Novelli *et al*., 1998) which is an early subtle effect of cadmium exposure as reported by research studies (Manca *et al*., 1991). Superoxide radical plays an active role in damaging liver and kidney of animals caused by cadmium toxicity (Novelli *et al*., 1998).

Gold, lead and other toxic metals are associated with heavy metal nephropathy which is a pathologic entity of the renal tubular epithelium of *Rattus* (rats). It is indicated acutely by the accidental death of cells induced by ischemia, subacutely by cortical fibrosis, and chronically by cytomegaly (characterized by the formation of enlarged cells) and karyomegaly (characterized by an enlarged cell nucleus). Eventually, a benign tumour develops in epithelial tissues, some of which becomes virulent (Payne and Saunders, 1978).

Lead poisoning combined with cadmium was diagnosed in sheep and horses of farmland within close range of non-ferrous metal smelters in a research study done in Baiyin of Gansu province in China. Anaemia with hypochromic and microcytic pattern was observed in affected animals. The research work proposed industrial activities to be the main culprit of environmental contamination by heavy metals and demonstrated that lead poisoning combined with cadmium caused ill health in sheep and horses in the region. The research study also suggested that concentration of harmful heavy metals in livestock must be checked on a periodic basis in order to assess deleterious effects of such contaminants on domestic animals (Liu, 2003).

Research studies have demonstrated that insectivorous bats bioaccumulate toxic heavy metals, resulting in their decline in recent decades which has become a focal point of public attention. Therefore, there is a growing concern regarding the study and conservation of bats all over the world (Pikula *et al*., 2010).

Presence of some toxic heavy metals such as lead (Pb), mercury (Hg), selenium (Se) and deficiency of some essential microelements such as chromium (Cr) was found in some wild species of north-east India. Behavioural studies suggested abnormalities in their performance such as salivation, lack of desire for food, constipation, photophobia (light sensitivity), tendency to dawdle in a circle, etc (Pandey and Madhuri, 2014).

Heavy metals may disturb the physiological and biochemical functions in fishes as shown by previous research studies. Cadmium, nickel, arsenic, inorganic arsenic, beryllium compounds and crystalline forms of silica have been reported to be carcinogenic in nature, leading to the development of cancer in fishes. Discharge of industrial effluents and products of boats and ships, such as heavy metals causes contamination of water bodies, ultimately inducing toxicity in marine animals.The tainted acquatic organisms used as sea food may put at risk the health of humans and animals resulting in reproductive and neurological disorders. Petroleum products trigger heavy metal pollution in acquatic environment and are the most pertinent contaminants to aquatic ecotoxicology. Different pestilent symptoms in experimental animals have been studied and are linked with exposure to crude oil and its derivatives (Govind *et al*., 2014).

Liver, kidney, spleen, muscle and lung samples of goats reared in the domain of an industrial area of West Bengal, India were analyzed in a research study for the manifeatation of disease. Analysis of blood of goats in the polluted site showed significantly ($P < 0.01$) decreased levels of total erythrocytes, total leucocytes, haemoglobin, total protein and packed cell volume compared with the reference site. While creatinine, serum glucose, aspartate amino transferase, alkanline phosphatase and alanine were remarkably ($P < 0.05$ to $P < 0.01$) increased. Cells and tissue examination of goats revealed various pathological alterations including necrosis of hepatocytes, degeneration, focal haemorrhages and clogging in liver; renal tubular vacuolation, deterioration, and tubulitis (presence of inflammatory cells in tubular wall) in kidney; diffuse haemorrhage and distention of blood vessels in lungs. It was proposed that notable increase in toxic heavy metal concentrations in several visceral organs of animals occur as a result of their natural exposure to these hazardous heavy metals leading to various pathological changes in the tissues of exposed animals (Kar *et al*., 2015).

Conclusion

Exposure to heavy metals in wild animals has remained a focal subject in several experminemtal studies. However, there is a lack of information concerning heavy metals exposure in domestic animals who share a similar habitat with humans and are inescapably exposed to the same destructive pollutants. Also, research studies are required on animals which are kept in protected areas. Regarding Pakistan, more persuasive research studies must be carried out on the use of animals as biomonitoring tool to assess both the health status of animals in particular and the environment in general.

References

Agarwal, S. K. (2009). *Heavy metal pollution* (Vol. 4). APH publishing.

Damek-Poprawa, M. & Sawicka-Kapusta, K. (2003). Damage to the liver, kidney, and testis with reference to burden of heavy metals in yellow-necked mice from areas around steelworks and zinc smelters in Poland. *Toxicology*, *186*(1-2), 1-10.

Duruibe, J. O., Ogwuegbu, M. O. C. and Egwurugwu, J. N. (2007). Heavy metal pollution and human biotoxic effects. *International Journal of physical sciences*, *2*(5), 112-118.

Falandysz, J., Szymczyk–Kobrzyńska, K., Brzostowski, A., Zalewski, K. and Zasadowski, A. (2005). Concentrations of heavy metals in the tissues of red deer (Cervus elaphus) from the region of Warmia and Mazury, Poland. *Food additives and contaminants*, *22*(2), 141-149.

Farlex, I. (2005). Definition: environment, the free dictionary.

Filistowicz, A., Dobrzański, Z., Przysiecki, P., Nowicki, S. and Filistowicz, A. (2011). Concentration of heavy metals in hair and skin of silver and red foxes (Vulpes vulpes). *Environmental monitoring and assessment*, *182*(1-4), 477-484.

Gdula-Argasińska, J., Appleton, J., Sawicka-Kapusta, K. and Spence, B. (2004). Further investigation of the heavy metal content of the teeth of the bank vole as an exposure indicator of environmental pollution in Poland. *Environmental Pollution*, *131*(1), 71-79.

Govind, P., Madhuri, S. and Shrivastav, A. B. (2014). Fish Cancer by Environmental Pollutants.

Gupta, V. (2013). Mammalian feces as bio-indicator of heavy metal contamination in Bikaner Zoological Garden, Rajasthan, India. *Res. J. Animal, Veterinary and Fishery Sci*, *1*(5), 10-15.

He, Z. L., Yang, X. E. and Stoffella, P. J. (2005). Trace elements in agroecosystems and impacts on the environment. *Journal of Trace elements in Medicine and Biology*, *19*(2-3), 125-140.

Herawati, N., Suzuki, S., Hayashi, K., Rivai, I. F. and Koyama, H. (2000). Cadmium, copper, and zinc levels in rice and soil of Japan, Indonesia, and China by soil type. *Bulletin of environmental contamination and toxicology*, *64*(1), 33-39.

Hobbelen, P. H. F., Koolhaas, J. E. and Van Gestel, C. A. M. (2006). Bioaccumulation of heavy metals in the earthworms Lumbricus rubellus and Aporrectodea caliginosa in relation to total and available metal concentrations in field soils. *Environmental pollution*, *144*(2), 639-646.

Horsfall Jr, M., Horsfall, M. N. and Spiff, A. I. (1999). Speciation of heavy metals in inter-tidal sediments of the Okrika river system, Rivers State Nigeria. *Bulletin of the Chemical Society of Ethiopia*, *13*(1), 1-10.

Kannan, K., Agusa, T., Evans, T. J. and Tanabe, S. (2007). Trace element concentrations in livers of polar bears from two populations in Northern and Western Alaska. *Archives of environmental contamination and toxicology*, *53*(3), 473-482.

Kar, I., Mukhopadhayay, S. K., Patra, A. K. and Pradhan, S. (2015). Metal concentrations and histopathological changes in goats (Capra hircus) reared near an industrial area of West Bengal, India. *Archives of environmental contamination and toxicology*, *69*(1), 32-43.

Khan, S., Cao, Q., Zheng, Y. M., Huang, Y. Z. and Zhu, Y. G. (2008). Health risks of heavy metals in contaminated soils and food crops irrigated with wastewater in Beijing, China. *Environmental pollution*, *152*(3), 686-692.

Ling, W., Shen, Q., Gao, Y., Gu, X. and Yang, Z. (2008). Use of bentonite to control the release of copper from contaminated soils. *Soil Research*, *45*(8), 618-623.

Liu, Z. P. (2003). Lead poisoning combined with cadmium in sheep and horses in the vicinity of non-ferrous metal smelters. *Science of the Total Environment*, *309*(1-3), 117-126.

Ma, W. C. (1987). Heavy metal accumulation in the mole, Talpa europea, and earthworms as an indicator of metal bioavailability in terrestrial environments. *Bulletin of Environmental Contamination and Toxicology*, *39*(6), 933-938.

Manca, D., Ricard, A. C., Trottier, B. and Chevalier, G. (1991). Studies on lipid peroxidation in rat tissues following administration of low and moderate doses of cadmium chloride. *Toxicology*, *67*(3), 303-323.

Masindi, V. and Muedi, K. L. (2018). Environmental Contamination by Heavy Metals.

Massadeh, A., Al-Sharif, L., Raed, D. H. and Hassan, M. (2006). Analysis of lead levels in local Jordanian and imported sheep meat and organs using atomic absorption spectrometry. *Environmental monitoring and assessment*, *115*(1-3), 87-93.

McLaughlin, M. J., Zarcinas, B. A., Stevens, D. P. and Cook, N. (2000). Soil testing for heavy metals. *Communications in Soil Science and Plant Analysis*, *31*(11-14), 1661-1700.

Merian, E. (1991). Metals and their compounds in the environment. Occurrence, Analysis and Biological Relevance. UCH. *Weintrein–New York.*

Novelli, E. L., Vieira, E. P., Rodrigues, N. L. and Ribas, B. O. (1998). Risk assessment of cadmium toxicity on hepatic and renal tissues of rats. *Environmental research*, *79*(2), 102-105.

Pandey, G. and Madhuri, S. (2014). Heavy metals causing toxicity in animals and fishes. *Research Journal of Animal, Veterinary and Fishery Sciences*, *2*(2), 17-23.

Parmar, T. K., Rawtani, D. and Agrawal, Y. K. (2016). Bioindicators: the natural indicator of environmental pollution. *Frontiers in life science*, *9*(2), 110-118.

Payne, B. J. and Saunders, L. Z. (1978). Heavy metal nephropathy of rodents. *Veterinary pathology*, *15*(5_suppl), 51-87.

Peplow, D. (1999). *Environmental impacts of mining in eastern Washington*. University of Washington Water Center.

Phillips, D. J. (1977). The use of biological indicator organisms to monitor trace metal pollution in marine and estuarine environments—a review. *Environmental Pollution (1970)*, *13*(4), 281-317.

Pikula, J., Zukal, J., Adam, V., Bandouchova, H., Beklova, M., Hajkova, P. and Valentikova, L. (2010). Heavy metals and metallothionein in vespertilionid bats foraging over aquatic habitats in the Czech Republic. *Environmental toxicology and chemistry*, *29*(3), 501-506.

Rashed, M. N. and Soltan, M. E. (2005). Animal hair as biological indicator for heavy metal pollution in urban and rural areas. *Environmental Monitoring and Assessment*, *110*(1-3), 41-53.

Reglero, M. M., Monsalve-González, L., Taggart, M. A. and Mateo, R. (2008). Transfer of metals to plants and red deer in an old lead mining area in Spain. *Science of the Total Environment*, *406*(1-2), 287-297.

Ross, S. M. (1994). *Toxic metals in soil-plant systems*. John Wiley and Sons Ltd.

Seaward, M. R. D. and Richardson, D. H. S. (1989). Atmospheric sources of metal pollution and effects on vegetation. *Heavy metal tolerance in plants: Evolutionary aspects*, 75-92.

Sharma, R. K. and Agrawal, M. (2005). Biological effects of heavy metals: an overview. *Journal of environmental Biology*, *26*(2), 301-313.

Sonne, C., Dietz, R., Leifsson, P. S., Asmund, G., Born, E. W. and Kirkegaard, M. (2007). Are liver and renal lesions in East Greenland polar bears (Ursus maritimus) associated with high mercury levels?. *Environmental Health*, *6*(1), 11.

Zhang, M. K., Liu, Z. Y. and Wang, H. (2010). Use of single extraction methods to predict bioavailability of heavy metals in polluted soils to rice. *Communications in Soil Science and Plant Analysis*, *41*(7), 820-831.

Zocche, J. J., Leffa, D. D., Damiani, A. P., Carvalho, F., Mendonça, R. Á., Dos Santos, C. E. I. and de Andrade, V. M. (2010). Heavy metals and DNA damage in blood cells of insectivore bats in coal mining areas of Catarinense coal basin, Brazil. *Environmental research*, *110*(7), 684-691.