ESTIMATION OF ESTROGENIC HEAVY METALS IN WATER AND BODY OF A COMMON FISH (*Anabas sp.*) OF BORSOLA BEEL, GUWAHATI CITY, ASSAM, INDIA

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ABSTRACT

In Assam, a notable water body is the Borsola Beel situated near Rehabari area of Guwahati city, Assam, which was once upon a time famous for its fish diversity and was regarded as the Reserve Wetland in 2001. But, due to various anthropogenic activities of our fellow human beings the water body is degrading gradually. It has been assumed that there might be accumulation of Endocrine Disrupting Chemicals (EDCs) from the discharges of surrounding area in the water body as well as in the fish body. Some chemicals mimic a natural hormone, fooling the body by over- responding to the stimulus or responding at inappropriate times, some block the effects of a hormone from certain receptors while some directly stimulate or inhibit the endocrine system and cause overproduction or underproduction of hormones. Heavy metals such as Cadmium (Cd) & Lead (Pb) are reported to cause cancerous development also. Accumulation of these toxic heavy metals in body system is not favoured in nature. The present work aimed at the estimation of heavy metals (Pb & Cd) having alleged estrogenic properties in the Borsola Beel. The analysis part involves collection of water samples and of a common fish species (Anabas sp.) followed by analyzing them using Flame Atomic Absorption Spectrometry (FAAS). After the FAAS analysis, the estrogenic heavy metals concentration in water was found to be in the order Pb>Cd whereas in fish body tissues, especially in the Muscle tissue, Liver tissue, Ovary tissue, Kidney tissue and Blood serum was also found to be in the order Pb>Cd.

***Keywords**: Environmental estrogens, Environmental Endocrine Disrupting Chemicals (EDCs), Environmental estrogenic heavy metals and Estrogen mimics.*

INTRODUCTION

A living body requires various types of elements for carrying out various biological activities that are necessary for continuing the life process. Those “Elements” include inorganic and organic substances that are found easily in the environment. Out of them, the inorganic minerals like sodium, potassium, calcium, magnesium and heavy metals like iron, manganese, lead, mercury, chromium, cadmium, nickel, cobalt, beryllium copper *etc*., when present above the permissible limit are harmful. But, the ways to perceive them or the routes of exposure to these heavy elements are different. The pollution of the existing aquatic ecosystems by heavy metals has become a worldwide problem in recent years, as they are non-biodegradable and most of them show toxic effects in organisms. Among environmental pollutants, metals are of particular concern, due to their potential toxic effect and ability to bioaccumulate in aquatic environment (Censi *et al*., 2006). Heavy metal concentrations in aquatic ecosystems are usually monitored by measuring their concentrations in water, sediments and biota, which generally exist in low levels in water and attains considerable concentration in sediments and biota. Some heavy metals have been shown to have endocrine disrupting properties, interfering with the hypothalamic–pituitary–ovarian (HPO) axis. Heavy metals including both essential and non-essential elements have a particular significance in ecotoxicology, since they are highly persistent and all have the potential to be toxic to living organisms. Sediments are important sinks for various pollutants like pesticides and heavy metals and also play a significant role in the remobilization of contaminants in aquatic systems under favorable conditions and in interactions between water and sediment. Fish samples can be considered as one of the most significant indicators in freshwater systems for the estimation of metal pollution level (Begum *et al*., 2005). Heavy metals such as Cd and Pb have no known role in biological systems as they are toxic, even in trace amounts. Accumulation of heavy metals in a tissue is mainly dependent on water concentrations of metals and exposure period. Endocrine systems also referred to as hormone systems, in which hormones are released by glands that travel throughout the body, acting as chemical messengers. Hormones interface with cells that contain matching receptors in or on their surfaces and bind much like a key would fit into a lock. Although hormones reach all parts of the body, only target cells with compatible receptors are well equipped to respond. Once a receptor and a hormone bind, the receptor carries out the hormone's instructions by either altering the cell's existing proteins or turning on genes that will build a new protein. The female ovaries, male testes, pituitary, thyroid, thymus, parathyroid and adrenal glands are major constituents of the endocrine system. They are also referred to as the family of female sex hormones - estrone, estradiol (primary in women of reproductive age) and estriol produced mainly in the ovaries. Estrogens are hormones that are important for sexual and reproductive development, mainly in females. Estrogen is involved in the onset of puberty, secondary sex characteristics such as breasts, pubic and armpit hair. Estrogen also helps to regulate the menstrual cycle, controlling the growth of the uterine lining during the first part of the cycle. Environmental estrogen is much different from natural estrogen, in a sense that it is not produced by our endocrine system; environmental estrogens usually referred to as xenoestrogens, which literally means, “foreign estrogen,” comes from chemical components that exhibit some degree of estrogen-like activity that are obtained from the environment are thus known as “endocrine disruptors”. Estrogen dominance can lead to infertility, breast cancer, endometriosis, accelerated aging, fibromyalgia and many other pathologic issues. Environmental estrogen can be found in many things that our industrialized society relies so heavily upon, such as food, cleaning products, drugs, electronics, and many more. Even heavy metals and metalloids may have estrogenic activity, suggesting that these compounds are EDCs as well as more generalized toxicant. Some chemicals mimic a natural hormone, fooling the body by over-responding to the stimulus or responding at inappropriate times while some block the effects of a hormone from certain receptors. Some directly stimulate or inhibit the endocrine system and cause overproduction or underproduction of hormones. A variety of chemicals have been found to disrupt the endocrine systems of animals in laboratory studies, and there is strong evidence that chemical exposure has been associated with adverse developmental and reproductive effects on fish and wildlife in particular locations. Thus, from a physiological perspective, an endocrine-disrupting substance is a compound, either natural or synthetic, which, through environmental or inappropriate developmental exposures, alters the hormonal and homeostatic systems that enables the organism to communicate with and respond to its environment. EDCs often act via more than one mechanism. Some EDCs have mixed steroidal properties: for example, a single EDC may be both estrogenic and antiandrogenic. EDCs may be broken down or metabolized to generate subproducts with different properties. Moreover, many organs are targeted by sex steroids and are thereby vulnerable to endocrine disruption, including the hypothalamic-pituitary-gonadal system and non-reproductive tissues such as bone, muscle, and skin. For instance, EDC effects can involve altered ERα expression in hypothalamus and epididymis or uterus. Along with the direct influence of EDCs on estrogen or androgen actions, they can affect endogenous steroid production through negative and positive feedback, effects that may differ depending on developmental stage. Finally, there are coexisting mechanisms not directly mediated at the hypothalamic-pituitary-gonadal (HPG) system. For instance, reproductive dysfunction can result from thyroid disruption or nonspecific interference of reduced energy intake. Heavy metals are natural trace components of the aquatic environment, but their levels have increased due to industrial wastes, geochemical structure, agricultural and mining activities. Pb and Cd can be introduced into the aquatic environment through soil erosion, as atmospheric dust, by oil combustion, by both domestic and industrial landfills and by precipitation (Harte, 1991). Lead is ubiquitous in the aquatic ecosystem (Moriarity, 1990). It is bioaccumulative. It is present as inorganic or organic element. Lead can metabolize with calcium and subsequently accumulates in bone tissue (Seymore *et al*,.1995). It also accumulates in muscle, kidney and liver. The toxicity of Pb depends on the fish age, pH and water hardness (Nussey, 2000). Cd is known for its toxic effects in fish. It causes lesions in the testes and ovaries, reducing the consumption of oxygen by the gills. Pathological changes of kidney and intestinal tissues and lesions and skeletal deformities. The potential of these elements for increased biota exposure and health risk is troubling; therefore, they were listed as priority pollutants by the US Environmental Protection Agency All these sources of pollution affect the physiochemical characteristics of the water, sediment and biological components, and thus the quality and quantity of fish stocks. Excessive pollution of surface waters can lead to hazards in humans' health, either through drinking of water and/or consumption of contaminated fish. The concentration of metals is a function of species, and metals accumulate more in some fish tissues than in others. The obvious sign of highly polluted water, dead fish, is readily apparent, but the sublethal pollution might result only in unhealthy fish. Very low-levels of pollution may have no apparent impact on the fish itself, which would show no obvious signs of illness, but it may decrease the fecundity of fish populations, leading to a long-term decline and eventual extinction. Such low-level pollution could have an impact on reproduction, either indirectly via accumulation in the reproductive organs, or directly on the free gametes (sperm or ovum) which are released into the water. Cd is an estrogen mimic, experimented that female rats injected with Cd experienced earlier puberty onset, increased uterine weight, and enhanced mammary development. Cd treatment induced estrogen-regulated genes such as progesterone receptor and complement component C3. Cd-treated MCF-7 human breast cancer cells demonstrate many responses to Cd that are the same as those elicited by estrogen. Cd treatment stimulates MCF-7 cell growth, downregulates the estrogen receptor, stimulates the expression of the progesterone receptor, and stimulates estrogen response element in transient transfection experiments. Lead can activate estrogen receptor-dependent transcriptional expression assay and stimulate MCF-7 breast cancer cell growth (Cheryl A. Dyer, 2009).

Table 1: Maximum Permissible Limit (MPL) of Pb and Cd in Fish Tissues and Water:

Name of the heavy metals	MPL in fish tissues (in mg/kg)	MPL in water (in mg/L)
Pb	2.0	0.01
Cd	0.005	0.003

Source: FAO/WHO report, 2011.

Study Area: The study area was chosen to be the Borsola Beel located near Rehabari area of Guwahati city having site coordinates 26°10’44” N and 91°44’44” E, previously reported to have estrogenic heavy metals in it (Roy *et al.* 2011). The beel is one of the notable potential wetlands of Guwahati City that meets Bharalu River and finally falls into the mighty Brahmaputra River. Since the last decade, around 20 bighas of 85-90 bighas of land of Borsola Beel have been occupied for Human settlements, colleges & commercial buildings.

MATERIALS AND METHODS

Chemicals: All the chemicals were provided from North East Chemicals, Guwahati.

Collection of water: Water samples were collected into 500ml glass reagent bottles previously soaked in dilute nitric acid, and then rinsed with distilled de-ionized water. At the sampling site, the bottles were rinsed three times with lake water prior to filling. The water samples were collected just below the surface. One sample was collected from each sampling point. The bottles were sealed tightly, and covered with aluminium foil paper.

Acid Digestion of water sample: The collected water samples were then brought to the laboratory where the samples were acidified to make the pH less than or equal to 2, using 60% hydrochloric acid (Merck, Darmstadt, Germany), then stored in the dark at room temperature prior to analysis. For the analysis of estrogenic heavy metals (Pb & Cd), the water samples were subjected to nitric acid digestion. To 50ml of sample, 5ml of conc. nitric acid was added. The contents were then heated on a hotplate with the temperature of the hotplate not exceeding 160°C. Boiling of the mixture was continued until the volume was reduced to 10-20 ml. The contents of the beaker were then quantitatively transferred to a 100ml volumetric flask, and diluted to 50ml volume with deionized water. The samples were then filtered using Whattman filter paper and then subjected to analysis.

Estimation of Cd & Pb: After acid digestion the samples were then analyzed using flame atomic absorption spectrometry (FAAS) to obtain the "total metal" concentration, i.e., both dissolved and particulate, inorganically and organically bound metal.

Instrument used – Atomic Absorption Spectrophotometer
Company – Perkin Elmer, **Model –** 200A

After repeated trials of fishing, with a fishing net, a female *Anabas sp.* was collected of
Weight = 11gms, Length = 8cms, Breadth = 1cm and Height = 1.3cms

Scientific Classification:
Kingdom:- Animalia
Phylum:- Chordata
Class:- Actinopterygii
Order:- Perciformes
Family:- Anabantidae
Genus:- *Anabas sp.*

Collection of fish: Samples of fish were collected into plastic bucket with water to support the normal conditions, previously soaked in dilute nitric acid, and rinsed with distilled de-ionized water. Upon arrival at the laboratory, the fish were stored in a cool dry place in the water of the beel itself prior to analysis.

Acid Digestion of fish tissues: For the analysis of estrogenic heavy metals in muscle, kidney, ovary, liver tissues respectively and blood serum samples, each of muscle, kidney, ovary and liver tissues respectively from 1 fish collected (Anabas sp.) were dissected out using stainless steel knives and tweezers. Weighed tissues were transferred to aluminium foils, wrapped gently and stored in a fridge at 4°C before acid digestion. A mixture of perchloric acid and nitric acid is prepared at a ratio of 3:1 (18ml:6ml). The tissues are then transferred to the mixture and its contents were then heated on a hotplate with the temperature of the hotplate not exceeding 160°C. Boiling of the mixture was continued until the volume was reduced to 2 - 5 ml. The contents of the beaker were then quantitatively transferred to a 100ml volumetric flask, and diluted to 50ml volume with deionized water. The sample is further filtered using Whattman Filter paper and then subjected to analysis.

Centrifugation of blood: Blood was collected using a new sterilized needle rinsed with freshly prepared EDTA. This blood was then transferred to an eppendorf and stored in fridge at 4 degree celcius prior to centrifugation. Blood serum was then obtained from the blood sample by centrifuging it at a speed of 5000 rpm for 10mins. The supernatant was then collected and diluted upto 5ml with deionized distilled water.

Estimation: The obtained samples were then analyzed for Cd & Pb using FAAS.

RESULTS

Estimation of Cd and Pb in Water Samples of Borsola Beel using FAAS: A hollow cathode lamp containing Cd/Pb emits light from excited Cd/Pb atoms that produce the right mixture of wavelengths to be absorbed by any Cd/Pb atoms from the sample. In FAAS, the sample is atomized i.e., converted into ground state, free atoms in the vapour state and a beam of electromagnetic radiation emitted from excited Cd/Pb atoms is passed through the vaporized sample. Some of the radiation is absorbed by the Cd/Pb atoms in the sample. The greater the number of atoms there in the vapour, the more radiation is absorbed. The amount of light absorbed is proportional to the number of Cd/Pb atoms. A calibration curve is constructed by running several samples of known Cd/Pb concentration under the same conditions as the unknown. The amount of standard absorbances is compared with the calibration curve and this enables the calculation of the concentration of Cd/Pb in the unknown sample.

Table 2: Estimated values of Cd & Pb in Water of Borsola Beel.

Name of the sampling sites of the Borsola beel	MPL of Cd in water (in mg/L)	Estimated levels of Cd by FAAS (in mg/L)	MPL of Pb in water (in mg/L)	Estimated levels of Pb by FAAS (in mg/L)
1 Paltanbazar	0.003	0.010	0.01	*BDL
2 Chatribari	0.003	0.004	0.01	0.023
3 Chabipool	0.003	0.006	0.01	0.020

*BDL – Below Detectable Level

Average Cd deposition in water of Borsola Beel = 0.006mg/L and average Pb deposition is = 0.021mg/L.

Table 3: Estimated values of Cd & Pb in water of Botanical Garden, Gauhati University Campus taken as Control.

Name of the sampling site of Control	MPL of Cd in water (in mg/L)	Estimated levels of Cd by FAAS (in mg/L)	MPL of Pb in water (in mg/L)	Estimated levels of Pb by FAAS (in mg/L)
Botanical Garden, G.U. Campus	0.003	BDL	0.01	0.009

Estimation of Cd and Pb in Anabas sp. using FAAS (Borsola Beel): Four tissues namely, Ovary, Kidney, Liver and Muscle along with the blood serum obtained from the *Anabas sp.* were subjected to FAAS analysis for the detection of heavy metals Cd & Pb. Usually, FAAS analysis yield results in mg/L as the sample analyzed is in solution form. Thus, the obtained FAAS values in case of tissues needs to be converted to mg/kg which is done using the standard formula:

[Concentration of metals (mg/L) * Volume of sample (mL)] / Sample Weight (Kg) * 1000

Following obtained results were compared with the MPL in fish tissues standardized by WHO & FAO.

Table 4: Estimated and calculated values of Cd & Pb in tissues of Anabas sp. collected from Borsola Beel.

Name of the fish tissues of Anabas sp.	MPL of Cd (in mg/kg)	Estimated levels of Cd by FAAS (in mg/L)	Calculated value of Cd (in mg/kg)	MPL of Pb (in mg/kg)	Estimated levels of Pb by FAAS (in mg/L)	Calculated value of Pb (in mg/kg)
1 Ovary	0.005	0.024	10.000	2.0	0.021	8.75
2 Kidney	0.005	0.014	12.500	2.0	0.014	12.50
3 Liver	0.005	0.018	8.737	2.0	0.002	0.97
4 Muscle	0.005	0.009	4.687	2.0	0.024	12.50
5 Blood serum	0.005	0.009	--	2.0	BDL	--

Table 5: Estimated and calculated values of Cd & Pb in tissues of Anabas sp. collected from local fish market taken as Control.

Name of the fish tissues of *Anabas sp.*	MPL of Cd (in mg/kg)	Estimated levels of Cd by FAAS (in mg/L)	Calculated value of Cd (in mg/kg)	MPL of Pb (in mg/kg)	Estimated levels of Pb by FAAS (in mg/L)	Calculated value of Pb (in mg/kg)
1 Ovary	0.005	0.004	0.584	2.0	0.014	2.04
2 Kidney	0.005	BDL	BDL	2.0	BDL	BDL
3 Liver	0.005	0.0011	0.073	2.0	BDL	BDL
4 Muscle	0.005	0.0015	0.084	2.0	BDL	BDL
5 Blood serum	0.005	0.034	--	2.0	BDL	--

Statistical analysis: By comparing the estimated values with the MPL values we could obtain the following graphs.

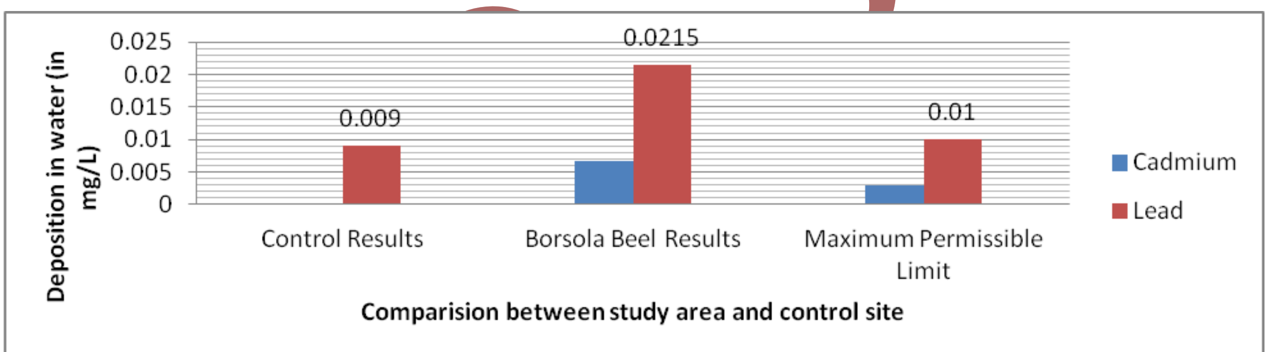

Fig.1: Graph depicting accumulation of Pb & Cd in water of Borsola Beel exceeding MPL.

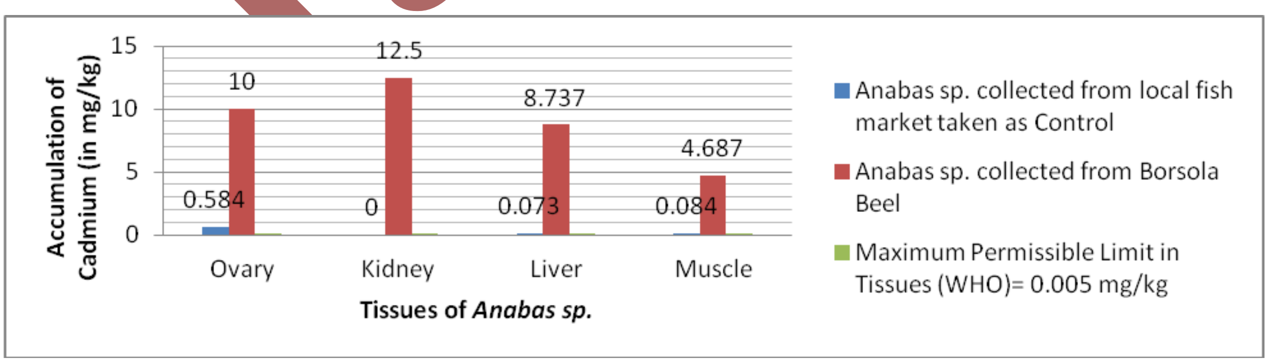

Fig. 2: Graph depicting accumulation of Cd in tissues of Anabas sp. of Borsola Beel exceeding MPL.

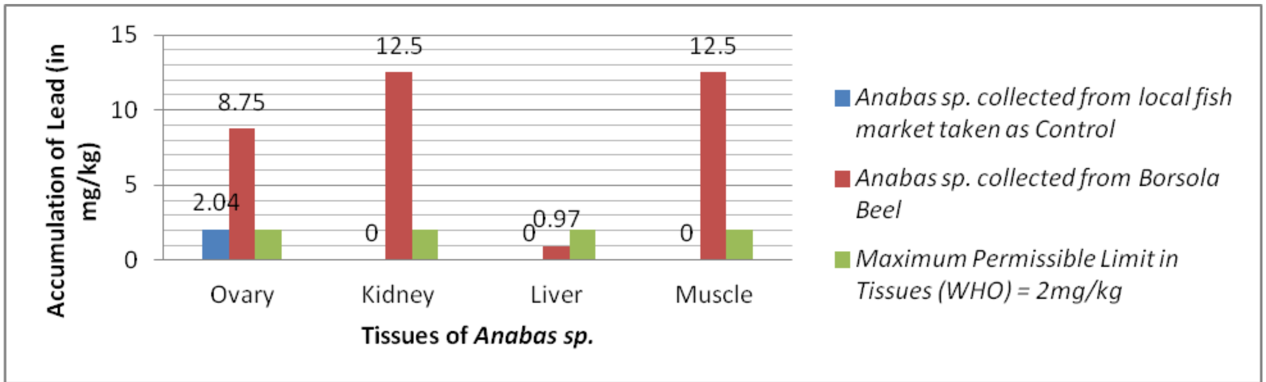

Fig. 3: Graph depicting accumulation of Lead in tissues of Anabas sp. of Borsola Beel exceeding maximum permissible limit.

DISCUSSION

The present study for the first time establishes the presence of Cd and Pb in tissues of fish body (*Anabas sp.*) of Borsola Beel. The water of the beel in which these fishes inhabit was evaluated by FAAS analysis to have accumulated a high level of Cd & Pb, above MPL level. In comparison to Cd accumulation, Pb is found in excesss which is alarming in nature since even 0.01mg/L is known to have high toxic effect (WHO 2011). After FAAS analysis, Cd was found to be present in the tissues of *Anabas sp.* above MPL (0.005mg/kg, by WHO & FAO, 2011) in the following order: Kidney>Ovary>Liver>Muscle. Pb was found to be present in the tissues of *Anabas sp.* above MPL (2.0mg/kg, by WHO & FAO, 2011) in the following order: Kidney>Muscle>Ovary>Liver. While in Blood Serum, Cd was found to be present slightly above the MPL level whereas Pb was found below detectable level (BDL). The prime consequence of such a situation may be biomagnification. It is a condition in which unnecessary and hazardous elements, specifically heavy metals, that exhibit estrogenic endocrine-disrupting property gets accumulated in the body of aquatic fauna (fishes of great food-value) due to their (heavy metals) accumulation and deposition in the water body wherein waterbody becomes the reservoir for dumping of settlement site-garbages, wastes, effluents of industries and construction-site disposables. On the other hand, those fishes enter the food-chain, constantly occurring in the ecosystem. Thus, the hazard gets multiplied during the process and so is termed as biomagnification. Moreover, biomagnifications can result when the water along with the fish get migrated from their present location to a totally different location, i.e.; from an affected region to non-affected region and deteriorate the features of the new location. As Borsola beel is inter-connected to the mighty river Brahmaputra by another water body-source, the Bharalu River, biomagnification might prove to be a threat. Moreover, the waterbody has been serving from a long period of time as the primary drainage system of that area and not just merely a reservoir of water. Constant dumping of wastes and landfill for settlement purposes has led to its massive shift from a one way-downstream waterbody to a flood-causing situation such that just a heavy rain causes havoc all over the city as the outlets get blocked due to manly activities. For this, the waterbody is in need of utmost action that could alter the situation or the havoc that it causes during rainy days. The municipality should take this information in person so that the right decision and required attention is provided to this man-made disaster.

CONCLUSION

Thus, the heavy metals – Cd & Pb levels in both the fishes and water body respectively might prove as a helpful tool in future assessment of the water body in order to its retrieval. Eventually, the studies on the above mentioned parameters has arrived us to a conclusion that the waterbody and its biodiversity is at high risk and needs immediate action in near future in order to control possible health hazards.

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