International Journal of Zoological Investigations Vol. 4, No. 2, 149-156 (2018)

International Journal of Zoological Investigations

Contents available at Journals Home Page: www.ijzi.net

ISSN: 2454-3055

Cadmium Attenuates Blood Calcium and Phosphate in the Indian Skipper Frog, *Euphlyctis cyanophlyctis*

Srivastav Ajai Kumar[1*], Srivastav Shilpi[1], Mishra Diwakar[2], Srivastav Sunil K.[1] and Suzuki Nobuo[3]

[1]Department of Zoology, DDU Gorakhpur University, Gorakhpur 273 009, India
[2]Department of Zoology, Govt. Girls P.G. College, Ghazipur 233001, India
[3]Noto Marine Laboratory, Institute of Nature and Environmental Technology, Kanazawa University, Ogi, Noto-cho, Ishikawa 927-0553, Japan

**Corresponding Author*

Received: 8th July, 2018
Accepted: 29th August, 2018

Abstract: Present study aimed at investigating effects of cadmium on blood electrolytes of Indian skipper frog, *Euphlyctis cyanophlyctis*. Frogs were subjected to cadmium chloride for short-term (18.432 mg/L i.e. 0.8 of 96 h LC_{50} value) and long-term (4.608 mg/L i.e. 0.2 of 96 h LC_{50} value). Frogs were sacrificed after 24, 48, 72 and 96 h in short-term and after 5, 10, 15 and 30 days in long-term experiment. Blood samples were analyzed for calcium and inorganic phosphate levels. Serum calcium levels of frogs exposed to cadmium for 24 h exhibit no alteration. After 48 h cadmium exposure level records a decrease which persists till 96 h. Serum inorganic phosphate levels of cadmium exposed frogs remain unaffected up to 48 h. Thereafter, values exhibit a progressive decrease from 72 h onwards. Frogs exposed to cadmium for 5 days exhibit no change in serum calcium. Thereafter, levels decrease progressively from day 10 till the end of the experiment (30 days 3). The serum phosphate levels of the frog following cadmium exposure remain unchanged till 10 days. Phosphate levels decrease progressively from 15 days onwards. Cadmium exposure leading to disturbances in serum calcium and phosphate levels may affect vital functions and even survival of frogs in nature.

Keywords: Cadmium, *Euphlyctis cyanophlyctis*, serum calcium, serum inorganic phosphate, Indian skipper frog

Introduction

Calcium is vital for living organisms and has been implicated in controlling a wide variety of physiological and biological functions (Srivastav *et al*., 2000; Michaels *et al*., 2015). It acts as a cofactor in various enzymic processes and couples stimulus-excitation reactions in muscle contraction and the secretion of exocrine and endocrine glands. It seems very difficult to mention a physiological process that does not, in one or another way, depend on calcium. Similarly, phosphorus is also required for intermediary metabolism (phosphorylated intermediates), genetic information (DNA and RNA), phospholipids, enzyme/protein components (phosphor-histidine, phosphoserine) and membrane structure (Norman and Litwack, 1987).

In vertebrates, the physiological control of these electrolytes is achieved by action on three major sites -- the intestine, by the control of absorption; the kidney, by the regulation of reabsorption from glomerular filtrate; and the bones, which act as a storage site for these electrolytes. Despite variations in intake and excretion, the coordination of the exchanges of these electrolytes at these three sites is under the strict control of three major hormones -- calcitonin, parathyroid hormone and vitamin D related steroids. These hormones interact with each other to maintain a constant concentration of these electrolytes. Calcitonin is a hypocalcemic hormone whereas parathyroid hormone and vitamin D metabolites are hypercalcemic.

The environment provides support to the organisms' life, but it also has significant impacts on their health as several hazardous toxicants are continuously being added every day to the environment. These toxicants accumulate in the edible species thus render these natural resources unfit for the consumption of human beings.

The impact of toxicants depends on its amount released to the environment and also to the sensitivity of the organisms – some species are more sensitive to a particular toxicant than others. Environmental toxicants may be short-lived (non-persistent) or long-lived (persistent even for decades). Short-lived toxicants affect only the immediate area of their release whereas long-lived toxicants may either restricted to the area of release or may be transported to other areas, accumulate in animal tissues, biomagnify in food chains, and provoke significant impacts on organisms health. The affected organism may survive but in their natural environment such influences can render them more vulnerable to predators, less able to compete with other species and less able to withstand the natural stresses.

In recent years, the study of amphibians has attracted interest among researchers due to the global amphibian population decline. Several causes have been proposed for such decline (Chambouvet *et al*., 2015; Michaels *et al*., 2015; Srivastav *et al.*, 2018).

Cadmium is a wide spread environmental contaminant. It enters in water reservoirs through natural (rocks and groundwater) and industrial sources. After entering water bodies cadmium may accumulate in aquatic organisms and plants. Although amphibians are a key component in ecosystem but the effects of cadmium have been poorly studied in amphibians (Selvi *et al*., 2003; Snodgrass *et al*., 2005; Sura *et al*., 2006; Mouchet *et al*., 2007; Sharma and Patino, 2008; Ilona *et al*., 2011; Gurkan *et al*., 2014). The cadmium accumulates in amphibian tissues (Othman *et al*., 2009; Enzymonye and Enuneku, 2012) and induces stress responses in frog skin (Simoncelli *et al*., 2015). There exists few studies regarding the interaction of cadmium with calcium homeostasis in mammals and birds. Also there are reports which describe the effects of certain toxicants on fish calcium regulation (Rai *et al*., 2008, 2009; Mishra *et al.*, 2004, 2005, 2011; Srivastav *et al*., 2009, 2010 a, b; Kumar *et al*., 2011 a, b; Prasad *et al.*, 2011 a, b, 2013). However, from amphibians there exists no study regarding the effects of cadmium on blood calcium and phosphate levels. Hence, in this study an attempt has been made to see the impact of cadmium chloride on the serum calcium and phosphate levels of an anuran amphibian, the Indian skipper frog *Euphlyctis cyanophlyctis*.

Materials and Methods

Laboratory reared frogs, *Euphlyctis cyanophlyctis* (both sexes; body wt. 12-17 g) were selected and acclimatized under natural photoperiod 11.58-12.38 and temperature 27.2±1.4 C for 15 days in 30 L all glass aquaria. The physico-chemical properties of water was pH 7.20 ± 0.1, Dissolved oxygen (mg/L) 7.95 ± 0.25, Hardness (mg/L) as $CaCO_3$ 167.06 ± 5.61 and Electrical conductivity (μmho/cm) 308.08 ± 66.12. Frogs were not fed 24 h before and during the experiment. Short-term and long-term experiments have been performed.

(i) Short-term exposure:

In this the frogs were subjected to 0.8 of 96 h LC_{50} value of cadmium chloride (18.432 mg/L). Frogs were kept in groups of 10 in 30 L media. Simultaneously, a control group (separate control group for each interval) was also used for comparison. Six frogs were killed on each time intervals from control and experimental groups after 24, 48, 72 and 96 h of exposure period.

(ii) Long-term exposure:

The frogs were subjected to 4.608 mg/L (0.2 of 96 h LC_{50} value) of cadmium chloride for 30 days. Simultaneously, a control group (separate control group for each interval) was also used for comparison. Six frogs from the control and experimental groups were sacrificed after 5, 10, 15 and 30 days of the toxicant treatment.

At each interval (in short- and long-term experiment) frogs were slightly anesthetized with ether and blood samples were collected by cardiac puncture. Blood samples thus collected were allowed to clot at room temperature. Sera were separated by centrifugation (at 3000 rpm) and kept at -20C until analyzed for serum electrolytes by using commercial diagnostic kits -- calcium (calcium kit, Sigma-Aldrich) and inorganic phosphate (Pointe Scientific, USA). All determinations were carried out in duplicates for each sample.

All data were presented as the mean ± SE of six specimens and Student's t test was used to determine statistical significance. In all studies, the experimental group was compared to its specific time control group.

Results

(a) Short-term cadmium chloride exposure (0.8 of 96 h LC_{50}):

The serum calcium levels of the frogs exposed to cadmium for 24 h exhibit no alteration. After 48 h cadmium exposure the level records a decrease. This response persists till the end of the experiment (96 h) (Fig. 1).

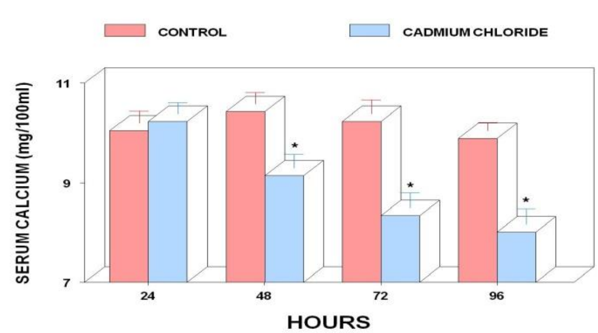

Fig. 1. Serum calcium levels of short-term cadmium chloride treated *Euphlyctis cyanophlyctis*. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences (P< 0.05) from control.

Up to 48 h following exposure of *Euphlyctis cyanophlyctis* to cadmium the serum inorganic phosphate levels remain unaffected. Thereafter, the values exhibit a progressive decrease from 72 h onwards (Fig. 2).

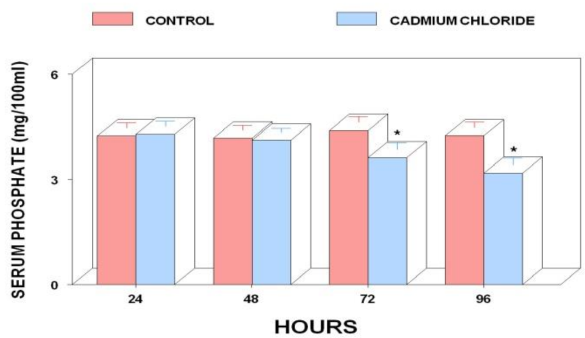

Fig. 2. Serum phosphate levels of short-term cadmium chloride treated *Euphlyctis cyanophlyctis*. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences (P< 0.05) from control.

(b) Long-term cadmium chloride exposure (0.2 of 96 h LC_{50}):

The frogs exposed to cadmium for 5 days exhibit no change in the serum calcium level. Thereafter, the levels decrease progressively from day 10 till the end of the experiment (30 days; Fig. 3).

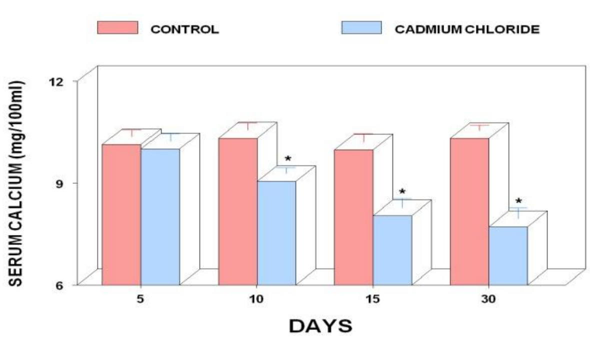

Fig. 3. Serum calcium levels of long-term cadmium chloride treated *Euphlyctis cyanophlyctis*. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences (P< 0.05) from control.

The serum phosphate levels of the frog following cadmium exposure remain unchanged till 10 days. The phosphate levels decrease progressively from 15 days onwards (Fig. 4).

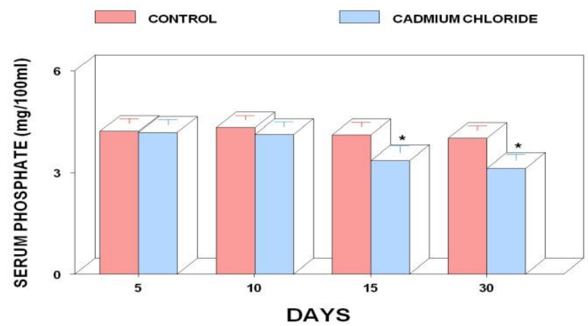

Fig. 4. Serum phosphate levels of long-term cadmium chloride treated *Euphlyctis cyanophlyctis*. Values are mean ± S.E. of six specimens. Asterisk indicates significant differences (P< 0.05) from control.

Discussion

In this study cadmium treatment caused hypocalcemia and hypophosphatemia in frogs. There exists no report regarding the effect of toxicants on the blood/serum calcium and phosphate levels of frogs, hence this study is first report regarding the cadmium induced hypocalcemia and hypophosphatemia in frogs. The observed hypocalcemia in cadmium exposed frogs is in conformity with the reports of other investigators who have observed similar effects after exposure of cadmium to fish (Larsson *et al*., 1981; Pratap *et al*., 1989; Rai and Srivastav, 2003; Rai *et al*., 2009), rabbits (Kenny, 1966) and rats (Tripathi and Srivastav, 2011). After treatment with various toxicants hypocalcemia has also been noticed in amphibian -- chlorpyrifos (Srivastav *et al*., 2018) and the fish–deltamethrin (Srivastav *et al*., 1997, 2010 a), cypermethrin (Mishra *et al*., 2011), lead (Rai *et al*., 2010, 2013) and botanical pesticides (Kumar *et al*., 2011 a, b; Prasad *et al*., 2011 a, b, 2013). However, few investigators have reported either no effect (Oner *et al*., 2008; Velisek *et al*., 2009) or increased blood calcium levels (Sharma *et al*., 1982; Suzuki *et al*., 2006) after treatment of fish to various toxicants.

Hypophosphatemia has been recorded in the foregoing study following cadmium treatment to frogs. In past hypophosphatemia has been noticed after treatment with toxicants to ampbhibian (chlorpyrifos- Srivastav *et al.*, 2018), fish (cadmium – Rai and Srivastav, 2003; deltamethrin – Srivastav *et al*., 1997; azadirachtin – Kumar *et al*., 2011 a; *Euphorbia tirucalli* – Kumar *et al*., 2011 b; *Nerium indicum* –Prasad *et al*., 2011 b), chicken (gammabenzene hexachloride and quinalphos – Agarwal *et al*., 2009) and rats (cadmium – Tripathi and Srivastav, 2011; chlorpyrifos – Tripathi *et al*., 2013).

The decrease in blood electrolytes of frogs after cadmium treatment could be attributed to the degeneration of kidney tubules. Degeneration of kidney tubules have been noticed after toxicant treatment to amphibians (Hanafy and Soltan, 2007), fish (Srivastava *et al*., 1990; Akram *et al*., 1999) and mammals (Chmielnicka *et al*., 1989; Prozialeck *et al*., 2009; Tripathi and Srivastav, 2010). Renal lesion may cause toxicant induced hyperfiltration in the kidney thus resulting into increased urinary efflux of these electrolytes (Chmielnicka *et al*., 1989; Prozialeck *et al*., 2009). In cadmium exposed women increased calciuria has been noticed (Schutte *et al*., 2008). In past few investigators have also attributed degenerative changes in renal tubules as one of the main causes of hypocalcemic responses in cadmium exposed fishes (Koyama and Itazawa, 1977; Roch and Maly, 1979; Larsson *et al*., 1981; Haux and Larsson, 1984). Earlier it has been opined that lead induced ionoregulatory toxicity in rainbow trout, particularly the disturbance of Ca^{2+} homeostasis, is not exclusively a branchial phenomenon, but is in part a result of disruption of ionoregulatory mechanisms at the kidney (Patel *et al*., 2006).

Celvero *et al*. (1998) reported cadmium induced larval teratogenic and developmental abnormalities in amphibians. It also induced increased micronuclei (genotoxicity test) in *Xenopus laevis* larvae (Houchet *et al*. 2007) and developmental delay with decline in survival of *Bufo* tadpoles (James and Little, 2003).

Conclusion

It is concluded that cadmium chloride exposure adversely affects the blood electrolytes of the frog, thus causing physiological imbalances which might affect normal vital functions, growth rate, reproduction and also their survival in nature. These effects could be considered as one of the factor causing amphibian population decline.

References

Agarwal S, Batra M and Chauhan S. (2009) Effects of gamma benzene hexachloride and quinalphos on serum calcium and phosphorus levels in experimentally fed broiler chickens. Journal Immunology Immunopathology 11: 57-59.

Akram M, Hafeez MA and Nabi G. (1999) Histopathological changes in the kidney of a freshwater cyprinid fish, *Barilius vagra*, following exposure to cadmium. Pakistan Journal Zoology 31: 77-80.

Celevro F, Campani S, Ragghianti M, Bucci S and Mancino G. (1998) Test of toxicity and teratogenicity in biphasic vertebrates treated with heavy metals (Cr^{3+}, Al^{+3}, Cd^{2+}). Chemosphere 37: 3011-3017.

Chambouvet A, Gower DJ, Jirku M, Yabsley MJ, Davis AK, Leonard G, Maguire F, Doherty-Bone TM, Bittencourt-Silva GB, Wilkinson M and Richards TA. (2015) Cryptic infection of a broad taxonomic and geographic diversity of tadpoles by *Perkinsea protists*. Proceedings National Academy Sciences USA, http://www.pnas.org/cgi/doi/10.1073/pnas.1500163112

Chmielnicka J, Halatek T and Jedlinska U. (1989) Correlation of cadmium-induced nephrology and the metabolism of endogenous copper and zinc in rats. Ecotoxicology Environmental Safety 18: 268-276.

Ezemonye LT and Enuneku AA. (2012) Hepatic bioaccumulation of cadmium in the crowned bullfrog, *Hopplobatrachus occipitalis* and flat backed toad, *Bufo maculatus.* International Journal Aquatic Science. 3: 16-22.

Gurkan M, Cetin A and Hayretdag S. (2014) Acute effect of cadmium in larvae of green toad, *Pseudepidalea variabilis* (Pallas 1769). Arhiv Za Higijenu Rada I Toksikologiju 65: 301-309.

Hanafy, S and Soltan ME. (2007) Comparative changes in absorption, distribution and toxicity of copper and cadmium chloride in toads during the hibernation and the role of vitamin C against their toxicity. Toxicological Environmental Chemistry 89:. 89-110.

Haux C and Larsson A. (1984) Long term sublethal physiological effects on rainbow trout, *Salmo gairdneri*, during exposure to cadmium and after subsequent recovery. Aquatic Toxicology 5: 129-142.

James SM and Little EE. (2003) The effect of chronic cadmium exposure on American toad (*Bufo americanus*) tadpoles. Environmental Toxicological Chemistry. 22: 377-80.

Ilona ES, Anush ST and Yury PP. (2011) Effect of molybdenum, chrome and cadmium ions on metamorphosis and erythrocytes morphology of the marsh frog *Pelophylax ridibundus* (Amphibia: Anura). Journal Environmental Science Technology 4: 172-181.

Kenny A. (1966) Hypocalcemia in experimental cadmium poisoning. British Journal Industrial Medicine 23: 313-317.

Koyama J and Itazawa Y. (1977) Effects of oral administration of cadmium on fish. I. Analytical results of the blood and bones. Bulletin Japanese Society Science Fish 43: 523-526.

Kumar A, Prasad M, Mishra D, Srivastav SK and Srivastav Ajai K. (2011 a) Botanical pesticide, azadirachtin attenuates blood electrolytes of a freshwater fish *Heteropneustes fossilis*. Pesticide Biochemistry Physiology 99: 170-173.

Kumar A, Prasad M, Mishra D, Srivastav SK and Srivastav Ajai K. (2011 b) Effects of *Euphorbia tirucalli* latex on blood electrolytes (calcium and phosphate) of a freshwater air-breathing catfish *Heteropneustes fossilis.* Toxicological Environmental Chemistry 93: 585-592.

Larsson A, Bengtsson BE and Haux C. (1981) Disturbed ion balance in flounder *Platichthys flesus* L., exposed to sublethal levels of cadmium. Aquatic Toxicology 1: 19-35.

Michaels CJ, Antwis RE and Preziosi RF. (2015) Impacts of UVB provision and dietary calcium content on serum vitamin D_3, growth rates, skeletal structure and coloration in captive oriental fire-billed toad (*Bombina orientalis*). Journal Animal Physiology Animal Nutrition 99: 391-403.

Mishra D, Srivastav SK and Srivastav Ajai K. (2004) Plasma calcium and inorganic phosphate levels of a teleost *Heteropneustes fossilis* exposed to metacid50. Malaysian Applied Biology. 33: 19-25.

Mishra D, Srivastav SK and Srivastav Ajai K. (2005) Effects of the insecticide cypermehrin on plasma calcium and ultimobranchial gland of the teleost *Heteropneustes fossilis*. Ecotoxicology Environmental Safety. 60:193-197.

Mishra D, Rai R, Srivastav SK and Srivastav Ajai K. (2011) Histological alterations in the prolactin cells of a teleost, *Heteropneustes fossilis* after exposure to cypermethrin. Environmental Toxicology 26: 359-363.

Mouchet F, Gauthier L, Baudrimont M, Gonzalez P, Mailhes C, Ferrier V and Devaux A. (2007) Comparative evaluation of the toxicity and genotoxicity of cadmium in amphibian larvae (*Xenopus laevis* and *Pleurodeles waltl*) using the comet assay and the micronucleus test. Environmental Toxicology 22: 422-435.

Norman AW and Litwack G. (1987) Hormones. Pp. 355-396, Academic Press Inc., New York.

Oner M, Atli G and Canli M. (2008) Changes in serum biochemical parameters of freshwater fish *Oreochromis niloticus* following prolonged metal (Ag, Cd, Cr, Cu, Zn) exposures. Environmental Toxicology Chemistry.27:360-366.

Othman MS, Khonsue W, Kitana J, Thirakhupt K, Robson MG and Kitana N. (2009) Cadmium accumulation in two population of Rice Frog (*Fejervarya limnocharis*) naturally exposed to different environmental cadmium levels. Bulletin Environmental Contamination Toxicology 83: 703-707.

Patel M, Rogers JT, Pane EF and Wood CM. (2006) Renal responses to acute lead waterborne exposure in the freshwater rainbow trout (*Oncorhynchus mykiss*). Aquatic Toxicology 30: 362-371.

Prasad M, Kumar A, Srivastav SK and Srivastav Ajai K. (2011a) *Euphorbia royleana*, a botanical pesticide affects ultimobranchial gland of a catfish, *Heteropneustes fossilis*. Egyptian Journal Biology 13: 14-20.

Prasad M, Kumar A, Mishra D, Srivastav SK and Srivastav Ajai K. (2011 b) Alterations in blood electrolytes of a freshwater catfish *Heteropneustes fossilis* in response to treatment with a botanical pesticide, *Nerium indicum* leaf extract. Fish Physiology Biochemistry 37: 505-510.

Prasad M, Kumar A, Srivastav SK and Srivastav Ajai K. (2013) *Nerium indicum*, a botanical pesticide affects ultimobranchial gland of a teleost, *Heteropneustes fossilis*. Environmental Toxicology. 28: 661-665.

Pratap HB, Fu H, Lock RAC.and Wendelaar Bonga SE. (1989) Effect of water borne and dietary cadmium on plasma ions of the teleost *Oreochromis mossambicus* in relation to water calcium level. Archives Environmental Contamination Toxicology 18: 568-575.

Prozialeck WC, Edwards JR, Vaidya VS and Bonventre JV. (2009) Preclinical evaluation of novel urinary biomarkers of cadmium nephrotoxicity. Toxicology Applied Pharmacology 238: 301-305.

Rai R and Srivastav Ajai K. (2003) Effects of cadmium on the plasma electrolytes of a freshwater fish *Heteropneustes fossilis*. Journal Ecophysiology Occupational Health 3: 63-70.

Rai R, Mishra D, Srivastav SK and Srivastav Ajai K. (2008) Acute toxicity of cadmium against catfish, *Heteropneustes fossilis* (Siluriformes: Heteropneustidae) in static renewal bioassays. Ethiopian Journal Biological Science. 7: 185-191.

Rai R, Mishra D, Srivastav SK and Srivastav Ajai K. (2009) Ultimobranchial gland of a freshwater teleost, *Heteropneustes fossilis* in response to cadmium treatment. Environmental Toxicology 24: 589-593.

Rai R, Mishra D, Srivastav SK, Suzuki N and. Srivastav Ajai K. (2013) Effects of lead nitrate on histo-cytological alterations of corpuscles of Stannius of stinging catfish, *Heteropneustes fossilis*. Iranian Journal Toxicology 7: 823-830.

Roch M and Maly EJ. (1979) Relationship of cadmium induced hypocalcemia with mortality in rainbow trout (*Salmo gairdneri*) and the influence of temperature on toxicity. Journal Fisheries Research Board Canada 36: 1297-1303.

Schutte R, Nawrot TS, Richart T, Thijs L, Vanderschueren D, Kuznetsova T, Van Hecke E, Roels HA and Staessen JA. (2008) Bone resorption and environmental exposure to cadmium in women: A population study. Environmental Hlth. Perspectives 116: 777-783.

Selvi M, Gui A and Yilmaz M. (2003) Investigation of acute toxicity of cadmium chloride ($CdCl_2$. H_2O) metal salt and behavioural changes it causes on water frog *Rana ridibunda*. Chemosphere 52: 259-263.

Sharma B and Patino R. (2008) Exposure of *Xenopus laevis* tadpoles to cadmium reveals concentration-dependent bimodal effects on growth and monotonic effects on development and thyroid gland activity. Toxicological Sciences 105: 51-58.

Sharma ML, Agarwal VP, Awasthi,AK and Tyagi SK. (1982) Hematological and biochemical characteristics of *Heteropneustes fossilis* under the stress of congo red (diphenyl diszabine pthionic acid). Toxicol. Letters 14: 237-240.

Simoncelli F, Belia S, Rosa ID, Paracucchi R, Rossi R, Porta G.L, Lucentini L and Fagotti A. (2015) Short term cadmium exposure induces stress response in frog (*Pelophylax bergeri*) skin organ culture. Ecotoxicology Environmental Safety 122: 221-229.

Snodgrass JW, Hopkins WA, Jackson BP, Baionno JA.and Broughton J. (2005) Influence of larval period on responses of overwintering green frog (*Rana clamitans*) larvae exposed to contaminated sediments. Environmental Toxicol. Chemistry 24: 1508-1514.

Srivastav Ajai K, Das VK, Srivastav SK and Suzuki N. (2000) Amphibian calcium regulation: Physiological aspects. Zoologica Poloniae 45: 9-36.

Srivastav Ajai K, Srivastav S, Srivastav SK and Suzuki N. (2018) Alterations in the Serum Electrolytes of the Indian Skipper Frog *Euphlyctis cyanophlyctis* caused by an organophosphate pesticide: Chlorpyrifos. Jordan J. Biol. Sci. 11:395-399.

Srivastav Ajai K, Srivastava SK and Srivastav SK. (1997) Impact of deltamethrin on serum calcium and inorganic phosphate of freshwater catfish *Heteropneustes fossilis*. Bulletin Environmental Contamination Toxicology 59: 841-846.

Srivastav Ajai K, Srivastava SK, Mishra D, Srivastav SK and Suzuki N. (2009) Effects of deltamethrin on serum calcium and corpuscles of Stannius of freshwater catfish, *Heteropneustes fossilis.* Toxicological Environmental Chemistry 91: 761-772.

Srivastav Ajai K, Srivastava SK, Mishra D and Srivastav SK. (2010 a) Deltamethrin-induced alterations in serum calcium and prolactin cells of a freshwater teleost, *Heteropneustes fossilis*. Toxicological Environmental Chemistry 92: 1857-1864.

Srivastav Ajai K, Srivastava SK, Tripathi S, Mishra D and Srivastav SK. (2010 b) Chlorpyrifos based commercial formulation: alterations in corpuscles of Stannius of catfish. International Journal Environmental Health 4: 323-332.

Srivastava SK, Tiwari PR and Srivastav Ajai K. (1990) Effects of chlorpyrifos on the kidney of freshwater catfish *Heteropneustes fossilis.* Bulletin Environmental Contamination Toxicol. 45: 748-751.

Sura P, Wrobel M and Bronowicka P. (2006) Season dependent response of the marsh frog (*Rana ridibunda*) to cadmium exposure. Folia Biologica 54: 159-165.

Suzuki N, Tabata MJ, Kambegawa A, Srivastav Ajai K, Shimada A, Takeda H, Kobayashi M, Wada S, Katsumata T and Hattori A. (2006) Tributylin inhibits osteoblastic activity and disrupts calcium metabolism through an increase in plasma calcium and calcitonin levels in teleosts. Life Sciences 78: 2533-2541.

Tripathi S and Srivastav Ajai K. (2010) Cytoarchitectural alterations in kidney of Wistar rat after oral exposure to cadmium chloride. Tissue Cell 43:131-136.

Tripathi S and Srivastav Ajai K. (2011) Alterations in the serum electrolytes, calcitonin cells and parathyroid gland of Wistar rat in response to administration of cadmium. Proc. Intern. Con. Environ. Pollution and Remediation Ottawa, Ontario, Canada, 17-19 August , Paper No. 126.

Tripathi S, Suzuki N and Srivastav Ajai K. (2013) Response of serum minerals (calcium, phosphate and magnesium) and endocrine glands (calcitonin cells and parathyroid gland) of Wistar rat after chlorpyrifos administration. Microscopy Research Technique. 76:. 673-678.

Velisek J, Svobodova Z and Piackova V. (2009) Effects of acute exposure to bifenthrin on some haematological, biochemical and histopathological parameters of rainbow trout (*Oncorhynchus mykiss*). Veterinarni Medicina 54: 131–137.