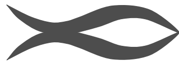

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RESEARCH PAPER

Differential Effects of Heavy Metals (Cadmium, Cobalt, Lead and Mercury) on Oocyte Maturation and Ovulation of the Catfish *Heteropneustes fossilis*: an *In Vitro* Study

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Abstract

The aim of the present study was to examine comparative effects of different concentrations of heavy metals such as cadmium (Cd), cobalt (Co), lead (Pb) and mercury (Hg) on catfish oocyte maturation and ovulation. Post-vitellogenic oocytes of the catfish *H. fossilis* were incubated with heavy metals at concentrations of 0.1, 1.0, 10 and 50ng/ml. Translucent follicles without germinal vesicle and opaque follicles with GV were scored after 4, 8, 16, and 24 h of incubation. Percentage GV breakdown (GVBD) was calculated as an index of oocyte maturation. The results of present investigation suggested that the effect of heavy metals on oocyte maturation and ovulation was in the order of Pb>Hg>Cd>Co. Pb and Hg showed significant increases (P<0.05) in GVBD and ovulation at all concentrations during 4 and 8hr of the incubation. However, there was a dose –dependent decrease at 16 and 24 h. Cd and Co showed decline in GVBD and ovulation at 16 and 24 h of the incubation. However, mercury and lead showed a stimulatory effect on ovulation at 16 and 24 h compared to control (P<0.05). Thus, these results suggest that catfish oocytes are differentially influenced by heavy metals and are resistant to them at certain levels.

Keywords: Heavy metals, catfish, ovary, oocyte maturation, ovulation.

Introduction

In teleost, as in other non-mammalian vertebrates, the principal events responsible for the enormous growth of oocytes are due to the accumulation of yolk proteins within their cytoplasm (Devlin & Nagahama, 2002). After the oocyte completes its growth it becomes ready for the next phase of oogenesis, i.e. the resumption of meiosis (completion of the first meiotic division followed by progression to metaphase II), which is accompanied by several maturational process in the nucleus and the cytoplasm of the oocyte. This process called oocyte maturation, occurs prior to ovulation and is a prerequisite for successful fertilization; it consists of the germinal vesicle break down (GVBD), chromosome condensation, assembly of the meiotic spindle and formation of the first polar body (Nagahama & Yamashita, 2008). The endocrine regulation of oocyte maturation and ovulation has been investigated most extensively in fishes (Nagahama, Yoshikuni, Yamashita & Nagahama, 1994; Thomas, Baer & White, 1994; Nagahama, 1997). Studies using well characterized *in vitro* as well as *in vivo* systems have revealed that oocyte maturation in fish is regulated by three mediators, gonadotropin (GTH and Luteinizing hormone, LH), maturation inducing hormone (MIH), and maturation promoting factor (MPF).

Endocrine disruptors have been reported to interfere with steroid hormone production or steroid mediating signaling in the gonads and eventually lead to serious damage of the reproductive organs and function. Fish inhabits practically all aquatic zones and are highly sensitive to changes in the environment. Hence, they play an important role in monitoring any kind of aquatic pollution and are widely used to evaluate the health of aquatic ecosystems. Heavy metals belong to undesirable substances in water environment. They are known for their ability of persistent accumulation in animal tissues. They can influence fish organism and disturb the process of reproduction (Thomas, 1993; Mukherjee, Kumar, & Chakraborti, 1994; Szczerbik *et al*, 2008). Cd is one of the most dangerous among heavy metals (Łukjanienko, 1974). Nandi, Gupta, Selvaraju, Roy and Ravindra (2010) has shown that culturing Buffalo (*Bubalus bubalis)* oocytes in different increasing concentrations of heavy metals for 24 hr had detrimental effects on cumulus

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expansion and oocyte maturation. Chronic exposure to pollutants like heavy metals leads to reproductive dysfunction in aquatic species (Choubey, Chaube & Joy, 2015). Heavy metals are not only highly toxic and persistent in nature but are also reported to have an endocrine disruptive potential (Zhu, Kusaka, Sato& Zhang 2000). Different heavy metals like cadmium, mercury, lead, arsenic, manganese and zinc are known to cause effect on endocrine system (Dyer, 2007; Luszczek-Trojnar *et al*., 2014). It has been reported that chronic exposure to cadmium influences reproductive disruption in fish, inhibiting induction of vitellogenin (Olsson *et al*., 1995), delaying oogenesis in brown trout (Brown *et al*., 1994) enhancing luteinizing hormone (LH) secretion and decreasing parameters og gonadosomatic index (GSI) and ovulation in Prussian carp (Szczerbik *et al*., 2006).

Heavy metals enter into various environmental matrices (air, water and soil) from a wide variety of natural and anthropogenic sources. All the sources of pollution affect the physicochemical characteristics of the water and biological components and thus the quality and quantity of fish. Fishes are one of the most widely distributed organisms in the aquatic environment and, being susceptible to heavy metal contamination (Khillare *et al*., 2017). As a result, large scale mortality of fishes has been observed due to discharge of heavy metals in natural water resources (FAO, 1996). Catfish *Heteropneustes fossilis,* which is an annual breeder and breeds during the monsoon rainfall (July–August). The fish is economically important for aquaculture in the Indian subcontinent and the flesh is highly priced, which is rich in protein but poor in lipid and fat content. At Varanasi due to heavy pollution in Ganges River and anthropogenic activities, river water is contaminated by heavy metal pollutants, which has severe impact on survivality of aquatic organisms specially fish population. Oocyte maturation and ovulation is the sensitive parameter for assessment of such pollutants. Thus, the objective of the present study was to examine the effect of heavy metals, cadmium (Cd), cobalt (Co), lead (Pb) and mercury (Hg) on catfish *H. fossilis* oocyte maturation and ovulation under *in vitro* conditions.

Materials and Methods

Chemicals

Cadmium Chloride ($CdCl_2$, Mol. Wt.- 183.32); Cobalt Chloride ($CoCl_2$, Mol. Wt. :- 237.93); Lead Nitrate ($Pb(NO_3)_2$, Mol.Wt.:-331.21) and Mercuric chloride ($HgCl_2$, Mol. Wt.:- 271.50) were purchased from Sisco Research laboratories (SRL) Pvt. Ltd. Mumbai, Maharashtra, India. All other chemicals were of analytical grade and purchased locally.

Animal Collection and Maintenance

The experiments were performed in accordance with the guidelines of Banaras Hindu University for experimentation in animals and all care was taken to prevent cruelty of any kind. Mature female catfish *Heteropneustes fossilis* (40-50 g) were purchased from local fish market in the pre-spawning phase (May-June) of the annual reproductive cycle. The gonado-somatic index (GSI) was 8.03±0.56. They were maintained in the laboratory under normal photoperiod (13.0L: 11.0D) and temperature (25±2º C) until used for experiments. The fish were fed goat liver daily *ad libitum*. A few fish were sacrificed randomly to determine the maturation of ovary. The fish containing ovaries filled with dark green post-vitellogenic follicles (oocyte diameter 1.0 mm) were used in the study.

Preparation of Incubation Medium and Test Compounds

The incubation medium consisted of (in grams) NaCl 3.74, KCl 0.32, $CaCl_2$ 0.16, $NaH_2PO_4{\cdot}2H_2O$ 0.10, $MgSO_4{\cdot}7H_2O$ 0.16 and glucose 0.40 in one L of triple distilled water (Goswami & Sundararaj, 1971). As phenol red (indicator of pH) is estrogenic, it was omitted in the preparation of the medium. The pH was adjusted to 7.5 with 1N sodium bicarbonate and autoclaved. Penicillin (200,000U) and streptomycin sulphate (200 mg) were added and filtered. The medium was stored at 4°C and was prepared fresh every week. Just before the incubation, $CdCl_2$, $CoCl_2$, $Pb(NO_3)_2$ and $HgCl_2$ were dissolved in the incubation medium to give different working concentrations (0.1, 1, 10, 50ng/mL).

Effects of Heavy Metals (Cd, Co, Pb and Hg) On GVBD and Ovulation

All instruments and glassware were sterilized. Gravid female *H. fossilis* were sacrificed by decapitation and ovaries were transferred to a Petri dish containing fresh cooled incubation medium. Round, dark green post-vitellogenic ovarian follicles (1mm diameter) were separated with fine brush and watchmaker's forceps. Batches of about 30-40 follicles were incubated in embryo cups containing 3mL incubation medium or medium containing test compound (0.1, 1, 10, 50ng/mL) at 24±2°C for 4, 8, 16 and 24 hrs. In each experiment, follicles from 5 fish ovaries were used and the incubations were done in triplicate from each ovary. The medium was changed 4 hourly and replenished with fresh medium containing required amount of the test compound. As controls, the follicles were incubated in medium containing the vehicle.

GVBD Scoring

At the end of the incubation (4, 8, 16 or 24 h), the follicles were cleared in a clearing solution (ethanol : acetic acid : formalin, 6:1:3; Trant & Thomas, 1988) and observed under a stereobinocular

microscope. Translucent follicles without germinal vesicle (GV) were scored. Percentage of GV breakdown (GVBD) was calculated from the total number of follicles incubated that underwent GVBD (Figure 1).

$$\% \text{ GVBD} = \frac{\text{Number of follicles that underwent GVBD}}{\text{Total number of follicles incubated}} \times 100$$

Ovulation Response

After the termination of the experiments, the follicles were examined under a stereo-binocular for scoring percentage ovulation. The intact follicles (unovulated) and oocytes without the follicular layer (ovulated) were counted separately. The percentage of ovulation was determined from the total number of the follicles incubated (Figure1).

$$\% \text{ Ovulation} = \frac{\text{Number of follicles that underwent Ovulation}}{\text{Total number of follicles incubated}} \times 100$$

Statistical Analysis

All experiments were repeated three times using follicles of the ovaries obtained from five fish. The difference between control and exposed groups were analyzed by Two way ANOVA followed by Tukey's test using SPSS statistical software. P values of less than 0.05 were considered significant (Table 1 and Table 2). Pearson correlation was done between heavy metal duration /concentration with oocyte maturation and ovulation (Table 3, Table 4, Table 5, and Table 6).

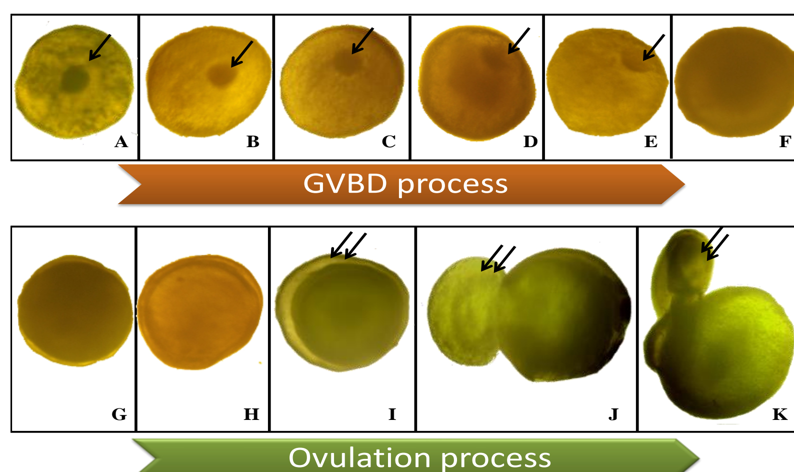

Figure 1. Showing the normal process of germinal vesicle breakdown (GVBD) and Ovulation.↙ - Germinal vesicle (GV), ↙↙Folicular envelope, A- GV in centre; B,C,- GV migrating towards periphery ; D- GV at periphery; E- GV breakdown started; F-GVBD complete ; G,H-Ovulation process starting; I- Oocyte hydrated showing detached follicular envelope; J-Follicular envelope detached; K-Ovulation complete.

Table 1. Statistical analysis using Two-way ANOVA followed by Tukey's test of effects of heavy metals on GVBD.

Two-way ANOVA ($P<0.001$); followed by Tukey's test ($P<0.05$)			
Heavy metals	$F_{duration}$	$F_{concentration}$	$F_{duration \times concentration}$
Cadmium	683.42	456.61	184.22
Cobalt	732.62	1408.1	158.08
Lead	1434.9	1530.8	121.94
Mercury	3949.3	781.3	322.6

Table 2. Statistical analysis using Two-way ANOVA followed by Tukey's test of effects of heavy metals on Ovulation.

Two-way ANOVA ($P<0.001$); followed by Tukey's test ($P<0.05$)			
Heavy metals	$F_{duration}$	$F_{concentration}$	$F_{duration \times concentration}$
Cadmium	9.871	41.491	10.393
Cobalt	26.03	52.818	12.126
Lead	440.26	100.41	59.085
Mercury	90.574	83.267	26.342

Results

Effects of Cadmium on GVBD and Ovulation

Incubation of the follicles with cadmium produced an overall significant effect on the percentage GVBD and ovulation after 4, 8, 16 and 24h (Figure 2A; P<0.001). Cd significantly increased GVBD in a concentration and duration dependent manner. It was maximum at 8hr and lowest at 24h at 10ng/mL dose. It decreased at 16 and 24h of incubation (*P*<0.05). On the other hand, % ovulation showed significant increase at 8 h with 0.1,1,10 and 50ng/mL. Further, at 16 and 24 h ovulation decreased significantly compared to control (Figure 2B; *P*<0.001). However, maximum rate of ovulation was limited to 6% which is just negligible.

Effects of Cobalt on GVBD and Ovulation

Incubation of the follicles with cobalt produced an overall significant effect on the percentage GVBD and ovulation after 4 ,8, 16 and 24h (Figure 3A; P<0.001). Co significantly increased GVBD in a concentration and duration dependent manner. It was maximum at 4 and 8h at 0.1ng/ml and lowest at 16 and 24h at 50ng/mL. There was significant concentration and duration dependent decrease (P<0.05). Cobalt treatment induced ovulation only upto 8%, which was maximum at 1ng/ml at 4 and 8 h. Further, there was decline due to death of follicles (Figure 3B; P<0.001).

Table 3. Pearson Correlation between heavy metals with duration of exposure for GVBD

GVBD	Duration (h)			
	4h	8h	16h	24h
Cd	-0.04095	0.342971	-0.07826	-0.35518
Co	-0.27876	-0.09469	-0.64449	-0.59659
Pb	0.630488	0.708183	0.630299	0.483135
Hg	0.103499	0.565914	-0.10214	-0.28969
Correlation is significant at P<0.05 level (2 tailed)				

Table 4. Pearson Correlation between heavy metals with duration of exposure for ovulation

GVBD	Duration (hr)			
	4h	8h	16h	24h
Cd	-0.42002	0.55177	0.883078	-0.10156
Co	-0.19768	0.47173	-0.10011	-0.10156
Pb	0.756954	0.67743	0.087686	0.196078
Hg	-0.2399	0.226299	0.642779	0.0883
Correlation is significant at P<0.05 level (2 tailed)				

Table 5. Pearson Correlation between heavy metals with different concentrations of exposure for GVBD

GVBD	Concentration (ng/mL)				
	Control	0.1ng/mL	1ng/mL	10ng/mL	50ng/mL
Cd	0.931143	0.835279	0.019667	-0.62763	-0.03707
Co	0.931143	-0.94121	-0.87356	-0.78165	-0.77388
Pb	0.931143	0.790449	0.814539	0.629724	0.481619
Hg	0.931143	-0.06847	-0.67795	-0.81567	-0.80802
	Correlation is significant at P<0.05 level (2 tailed)				

Table 6. Pearson Correlation between heavy metals with different concentrations of exposure for GVBD

GVBD	Concentration (ng/mL)				
	Control	0.1ng/mL	1ng/mL	10ng/mL	50ng/mL
Cd	0.232129	-0.74901	-0.79805	-0.24543	-0.149
Co	0.232129	-0.85889	-0.91141	-0.60741	-0.55971
Pb	0.232129	-0.79247	0.914674	0.355535	-0.17274
Hg	0.362085	-0.9092	0.065525	0.365561	0.121208
	Correlation is significant at P<0.05 level (2 tailed)				

Effects of Lead on GVBD and Ovulation

Incubation of the follicles with lead produced an overall significant effect on the percentage GVBD and ovulation after 4, 8, 16 and 24h (Figure 4A; P<0.001). Pb significantly increased GVBD in a concentration and duration dependent manner. It was maximum at 16hr at 10ng/ml and 50ng/ml and lowest at 4hr at 0.1ng/ml and 1ng/ml compared to control. There was significant concentration and duration

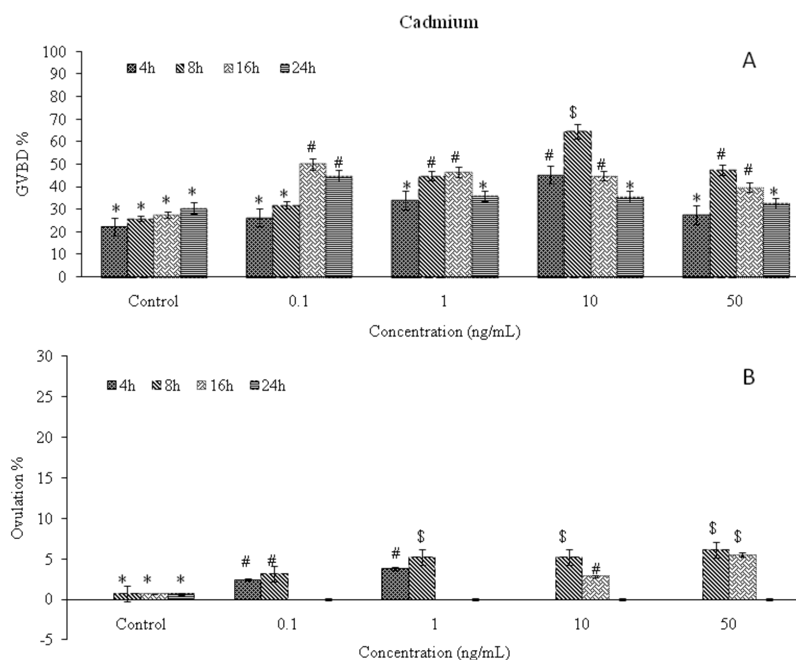

Figure 2. *In vitro* effects of different concentration of Cadmium (0.1, 1.0, 10.0 and 50.0 ng/ml) on GVBD (A) and ovulation (B) at 4, 8, 16 and 24 h in the catfish *Heteropneustes fossilis*. Data were expressed as mean±SEM and were analyzed by two way ANOVA, followed by Tukey's test (P<0.05). Groups with the same letter are not significantly different.

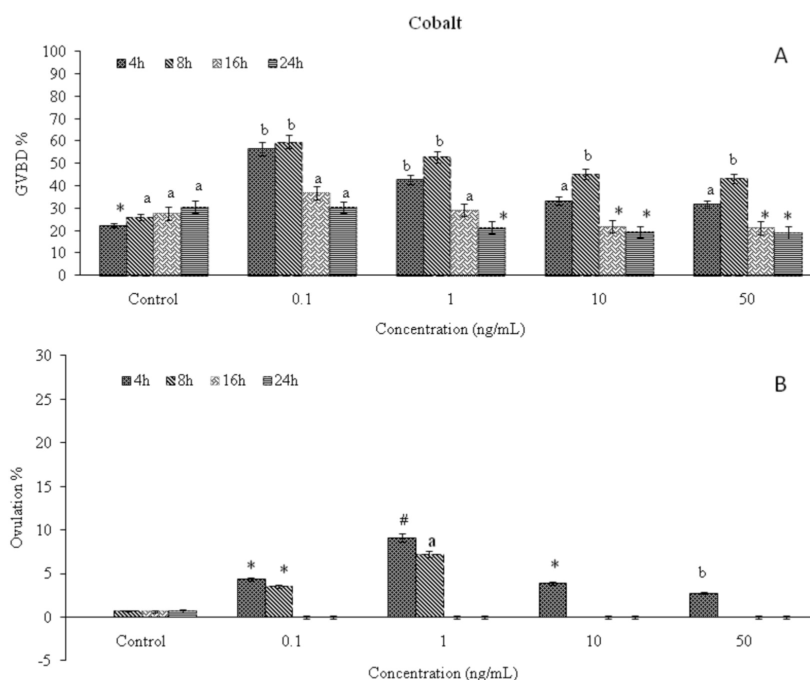

Figure 3. *In vitro* effects of different concentration of Cobalt (0.1, 1.0, 10.0 and 50.0 ng/ml) on GVBD (A) and ovulation (B) at 4, 8, 16 and 24hr in the catfish *Heteropneustes fossilis*. Data were expressed as mean ± SEM and were analyzed by two way ANOVA, followed by Tukey's test (P<0.05). Groups with the same letter are not significantly different.

dependent increase in GVBD upto 16h in all concentration groups and later on at 24h GVBD decreased. Lead treatment induced ovulation only upto 8%, which was maximum at 1ng/ml at 4 and 8 hr. Further, there was decline due to death of follicles (Figure 4B; *P<0.001*). Lead showed significant concentration dependent stimulation at all the durations as compared to control, maximum stimulation was seen at 16h.

Effects of Mercury on GVBD and Ovulation

Incubation of the follicles with mercury produced an overall significant effect on the percentage GVBD and ovulation after 4,8,16 and 24hr (Figure 5A; *P<0.001*). Hg significantly increased GVBD in a concentration and duration dependent manner. It was maximum at 4, 8, 16 and 24h at 0.1ng/ml and lowest at 16 and 24h at 50ng/mL. There was significant concentration and duration dependent decrease. Mercury treatment induced ovulation only upto 8%, which was maximum at 1ng/ml at 4 and 8 h. Further, there was decline due to death of follicles (Figure 5B; *P<0.001*). Mercury treated oocytes showed ovulation at all the durations, maximum effect seen at 8 and 16 h.

Correlation analysis resulted in mixed negative and positive correlation for all metal concentration with respect to duration for GVBD and ovulation. Lead exposure showed high positive correlation at 0.1 and 1.0 ng/mL for GVBD and ovulation. The order of correlation in GVBD with respect to concentration was as follows Pb>Cd>Hg>Co and for ovulation Pb>Hg>Cd>Co.

Discussions

The present studies demonstrate that exposure to heavy metals, i.e., cadmium, cobalt, mercury and lead, caused a decline in oocyte maturation and ovulation in a concentration and duration dependent manner. Heavy metals are endocrine disruptors with the ability to cause hormonal imbalances affecting various physiological processes such as reproduction. Chaube *et al*. (2010) showed that environmental Pb can be a potent endocrine disruptor affecting ovarian steroidogenesis, gametogenesis and ovulation, which may lead to adverse impact on fish reproduction and population density. In our study, heavy metals increased GVBD at low concentration and decreased at high concentration. Hwang, Kima, Kima, Leeb and Baeka (2010) showed that NP increased endogenous E_2 production at the vitellogenic stage and inhibited GVBD at the fully vitellogenic stage.

Studies in sheep (Leoni *et al*., 2002), cattle and mice (Yang, Xu & Wang, 2004) have provided evidence suggesting that exposures to cadmium disrupt oogenesis *in vitro*. Work on the effect of lead on mammalian oocytes *in vitro* is limited to a single study wherein lead was reported to affect the control of both meiosis arrest and meiosis resumption of the mouse oocyte in vitro, at least via the PKC pathway (Azaveri, Denys & Lefevre, 2006). Lead also induced changes in ovarian follicular development and maturation in mice (Junaid, Chowdhuri, Narayan, Shanker, & Saxena, 1997). This was the first report on the effect of heavy metal exposure on oocyte and embryo development *in vitro* in large ruminants. Both lead and cadmium caused a significant reduction in

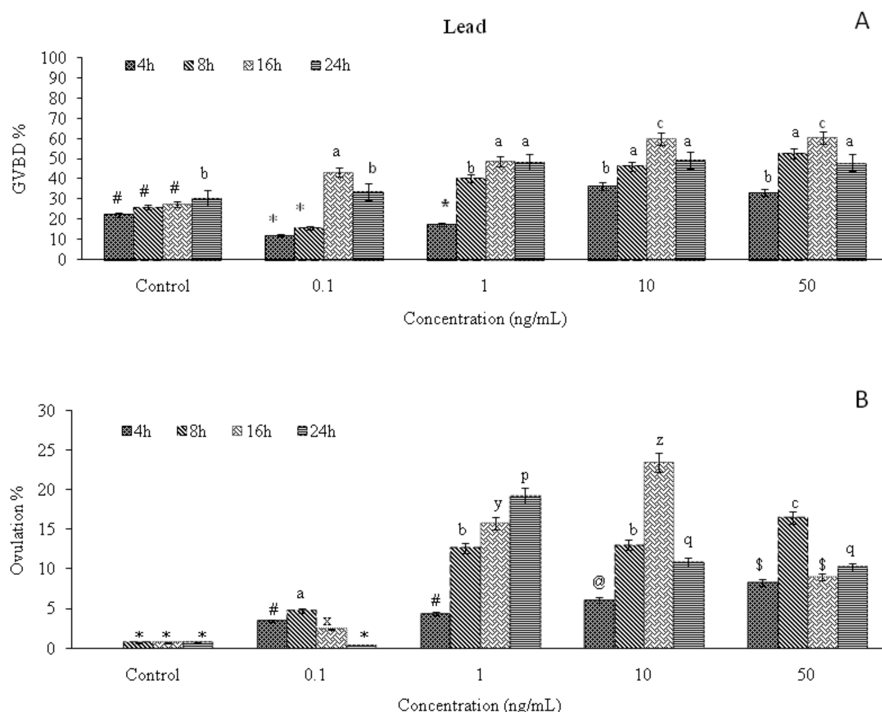

Figure 4. *In vitro* effects of different concentration of Lead (0.1, 1.0, 10.0 and 50.0 ng/ml) on GVBD (A) and ovulation (B) at 4, 8, 16 and 24hr in the catfish *Heteropneustes fossilis*. Data were expressed as mean ± SEM and were analyzed by two way ANOVA, followed by Tukey's test (P<0.05). Groups with the same letter are not significantly different.

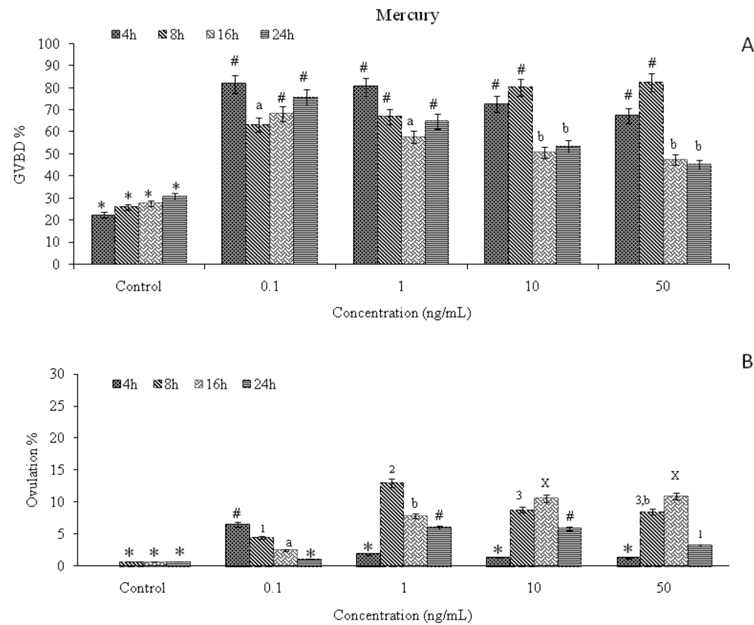

Figure 5. *In vitro* effects of different concentration of Mercury (0.1, 1.0, 10.0 and 50.0 ng/ml) on GVBD (A) and ovulation (B) at 4, 8, 16 and 24hr in the catfish *Heteropneustes fossilis*. Data were expressed as mean ± SEM and were analyzed by two way ANOVA, followed by Tukey's test (P<0.05). Groups with the same letter are not significantly different.

gonadotropin binding, which altered the steroidogenic enzyme activity of granulose cells (Nampoothiri & Gupta, 2006). Higher concentrations of cadmium inhibited activity of the P450scc gene (Smida, Valderrama, Agostini, Furlan & Chedrese, 2004), inhibited progesterone synthesis (Massanyi, Uhrin, Toman, Kovacik & Biro, 1999; Zhang & Jia, 2007), and facilitated changes in cell morphology and cell death (Smida *et al.,* 2004; Kondoh, Araragi, Sato, Higashimoto, Takiguchi & Sato, 2002). Thus, polluting substances, classified as endocrine disruptors, interfere with the endogenous hormone signaling system, leading to impairment of reproductive health (Caserta *et al*., 2011; Gupta *et al*., 2010; Zhang *et al.,* 2013).

Lead elicited biphasic effects on estradiol-17β, testosterone, and cortisol: stimulatory at lower concentrations and inhibitory at higher concentrations. The observation of such effects led the authors to make an unusual conclusion that in trace amounts, Pb may be beneficial (Chaube, Mishra, & Singh, 2010). The physiological significance of the stimulatory response of the low concentrations of Pb on the steroid levels is not clear. It may be that Pb like other essential metals (Cu, Zn, Mn, etc.) at very low concentrations may be beneficial, if not essential for fish metabolism (Patra, Swarup, Sharma, & Naresh, 2006; Sprocati *et al*., 2006). But it seems very doubtful because of the lack of any evidences for the physiological role of Pb.

Cadmium alters the secretory patterns of pituitary hormones (Lafuente, Cano, & Esquifino, 2003), stimulates the progesterone synthesis at low dose (Powlin, Keng, & Miller, 1997; Massanyi *et al*., 2000) and inhibits P4 synthesis at high doses (Jolibois, Shi, George, Henson & Anderson, 1999a; Jolibois *et al*., 1999b). Cd is shown to down regulate 11β-HSD by mimicking the ability of estrogen to attenuate the expression of this placental enzyme (Chowdhury & Arora, 1982). Smida *et al* (2004) reported the stimulatory effect of Cd on transcription of P450 side chain cleavage in porcine granulose cells resulting into increase in progesterone production.

Lead is shown to cause reduction in the expression of the steroidogenic acute regulatory protein (StAR), inhibition of LH secretion (Srivastava *et al*., 2004), increase in lipid peroxidation and ROS production (Kasperczyk *et al*., 2008; Hsu, Hsu, Liu, Chen, & Guo, 1998). In vivo exposure to lead suppressed circulating LH, FSH and estradiol without affecting progesterone in cynomolgus monkey (Foster, 1992). In utero exposure to Pb reduced ovarian primordial follicles in mice (Wide, 1985), adult mice exposed to pb by gavage for 60 days showed alteration in the population of small, medium and large ovarian follicles (Junaid *et al.,* 1997), and when injected it decreased primordial follicles and increased growing and atretic follicles (Taupeau, Poupon, Nome, & Lefevre, 2001).

It is known that Cd can act as a reproductive disrupter in animals (Singhal, Nijayvargiya, & Shukla, 1985). It was found to interfere with ovarian steroidogenesis in mammals (Paksy, Varga, Narav, Olajos & Folly, 1992) and fish (Mukherjee *et al.,* 1994). Cd was found to stimulate the spontaneous maturation of Prussian carp oocytes in our study. We suppose that such an effect could come from the chemical similarity of Cd to calcium. Calcium is a very important regulator of ovarian steroidogenesis (Veldhius & Klase, 1982; Tsang& Carnegie, 1983;

1984; Kleiss-San & Schuetz, 1987). The calcium channel blockers were shown to inhibit the forskolin (a direct adenyl cyclase activator) stimulated testosterone production in goldfish (Van Der Kraak, 1991). It was found that Cd affected calcium homeostasis by activating or inhibiting several calcium-related enzymes (Beyersmann & Hechtenberg, 1997).

Endocrine disruptive effect of cobalt was shown by Romani *et al*. (2014). HCG and cobalt chloride were shown to induce progesterone release, vascular endothelial growth factor release and mRNA expression in human luteal cells respectively. The estrogenicity of Hg was examined in MCF-7 cells. $HgCl_2$ stimulated both estrogen receptor dependent transcription and increased proliferation of MCF-7 cells (Choe *et al*., 2003). Mondal, Mukhopadhyay, and Bhattacharya (1997) reported that $HgCl_2$ stimulated the activity of 3β-HSD in oocyte of fish. Both *in vivo* and *in vitro* $HgCl_2$ treatment showed high rate of progesterone synthesis and low rate of conversion to 17β- Estradiol in the oocytes of *Channa punctatus.* Besides this Hg was also shown to cause reduction in sperm motility and sperm count (Chowdhury & Arora, 1982) and increase in plasma levels of T_4, TSH, Estrone and Estradiol (Abdelouahaba *et al*., 2008). Thus, in the present study, we showed that heavy metals can differentially modulate catfish oocyte maturation, ovulation. It may be related to direct or indirect effect of heavy metals on the oogenesis process, which ultimately results in reduced quantity and quality of catfish eggs and decline in population.

Conclusions

In conclusion, heavy metals inhibited or stimulated oocyte growth and development *in vitro* differentially. Toxicity of heavy metals in water may be the major threat for growth, development, reproduction and survival of the fishes as well as all the aquatic flora and fauna. This may have potential risk to catfish populations in the waters contaminated with heavy metals. The present study may serve as baseline data to monitor future anthropogenic activities in river water specific area. Further, mechanistic approach needs to be investigated

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