DISCUSSION

Pollution is the most serious of all environmental problems posing a major threat to the health and well being of people and animals (Holdgate, 1979) and water pollution has become a worldwide phenomenon (FEPA 1991). Varalakshmi and Ganeshmurthy (2010) have reported the presence of heavy metals such as chromium, cadmium, lead, iron and nickel in the waters of Nagavara lake. In the present study the waters of Nagavara lake was observed to contain heavy metals in the order lead > chromium > iron > zinc > cadmium and further, they were found to be above permissible limits as given by the Bureau of Indian Standards (BIS, 1991) and World Health organization (WHO, 1997). The above result may be attributed to the release of waste waters produced by the small scale industries located around it.

Heavy metals are non-biodegradable and hence tend to persist not only in the abiotic part of the environment but also enter living systems and undergo biomagnifications and bioaccumulation (Wicklund-Glynn, 1991). Heavy metals that accumulate in different organ of fish have hazardous impact on fish tissues (Kotze et al., 1999). Further, when such contaminated fishes are consumed by mammals, the metals enter the body and impair metabolism (Pourang et al., 2005). Ahmed and Bibi (2010) have reported the presence of heavy metals especially lead in various tissues including muscles of fishes living in polluted waters. In the present study the fish muscle of *Tilapia mossambicus* was found to accumulate heavy metal in the order chromium > lead > cadmium > zinc > iron. These results are in

Odum et al., (2001) reported body and organ weight to be influenced by diet. According to Mahaffey et al., (1991) cadmium and lead administered in combination may depress weight gain in rat. Moriyama et al., (2008) have opined decrease in body weight to be due to suppression of food and water intake. Results of the present study revealed a significant decrease (p<0.05) in the body weight and food intake of animals treated with different ratios of fish meal of both generations. This result was more pronounced in rats fed with higher ratios (1:3) and only fish meal fed rats. Decrease in food intake and body weight may be due to the presence of contaminants in the fish meal, leading to nutritional deficiency and period of exposure to different ratios of fish meal. Similar results have been reported in rodents by Morton, 2006; Arnold., 1986.

Nutritional deficiencies alter reproductive function in rats (Dikshith et al., 1988; Simonich et al., 1995 and Baligar et al., 2001). In the present study there was a significant decrease (p< 0.05) in the weight of ovary and uterus of rats fed with higher ratios of fish meal (1:3 and only fish meal) in both F1 and F2 generation. This reduction in ovarian weight may be due to non availability of gonadotropins that are essential for maintaining ovarian activity. Sejedmakic and Nihadaahmetovic (2012) have reported a decrease in uterine weight in rats treated with Zearalenone. Similar results are reported by Madhu et al., (2010) in atropine sulphate administered rats. In the present study, decrease in uterine weight is due to uterine decidualization which correlates with reduction in serum progesterone level. This result is in accordance with Ema et al., (1999). Similar results have also
been obtained in clomiphene citrate treated mice (Arun kumar and Pranab, 1995).

The status of estrous cycle and their inter conversion are mainly governed by hormones. Any change in these hormones would lead to changes in the cyclicity and impaired fertility (Freeman, 1994). In the present study, rats fed with different ratios of fish meal revealed, a decrease in the number of estrous cycles and a prolonged diestrous phase. These changes were more significant in F2 generation than in F1 generation rats, fed with higher ratios of fish meal (1:3 and only fish meal) when compared to control. Prolonged diestrous may be due to heavy metal toxicants that interferes with hormonal imbalance leading to altered ovarian function. Asmathabanu et al., (1997) and Math et al., (1998) have reported rats treated with organophosphate pesticide to show prolonged diestrous phase. Hence the persistence of the diestrous phase of the estrous cycle in the present study could be correlated with decrease in estradiol level. The results obtained in the present study are in accordance with the findings of Rao et al., (2002), Mattison and Nightingale, (1980) and Cooper et al., (1996).

Numerous endocrine disruptors that are released into the environment produce hormonal effects in animals and humans (Gray et al, 1999 and Guzelian, 1982). Some pesticides may interfere with the female hormonal function resulting in negative effects on the reproductive system by disrupting hormonal balance (Reini et al., 2006). In the present study estrogen, progesterone, FSH, LH and Prolactin was found to decrease with increasing ratios of fish meal. The results were more pronounced in F2 than F1 generation. This result may be due to atrophy of the granulosa cells resulting in changes in follicular morphology, inhibition of antral follicle, increase in
atretic follicle that causes reduction in estrogen level. These results are in accordance with Oduma et al., (1995) and Rani and Krishnakumari (1995). Reduction in progesterone level results in inhibition of ovulation. These results were in accordance with Ugwoke et al; (2004) and Uboh et al: (2007). Similar results have been reported by Ema and Miyawaki (2002) in diphenyltin treated rats. Present study showed reduction in FSH and LH in F1 and F2 generation which leads to suppression in secretion of hypothalamic gonadotropin releasing hormone (GnRH) from pituitary. This result is in accordance with Rai et al., (2004). Decrease in prolactin would likely lessen the response of circulating LH. Reduction in prolactin level in the present study is associated with metal stress which contributes to the disruption of hypothalamus-pituitary-axis. Similar results have also been obtained by Saez and Legaune (1996).

The ovary can be considered as an aggregate of three endocrine tissues, the stroma, the follicle and corpus luteum (Adkarprafulla et al., 2012). Anajulia et al., (2006) have noticed follicle numbers to decrease in flunitrazepam treated rats. In the present study the control rat ovary revealed a normal architecture exhibiting follicles in various stages of development and the rats fed with 1:1, 1:2 and 1:3 ratios of fish meal revealed the presence of degenerating growing follicles, loosely arranged stromal cells, disorganized ovum and numerous atretic follicles. In rats fed with only fish meal there was a decrease in the number of growing and mature follicles and an increase in the number of atretic follicles. These results were more pronounced in F2 generation rats than in F1. The increased number of atretic follicles and concomitant decrease in the number of pre antral and graffian follicle in fish meal treated rats may be due to the non availability
of the required amount of the extra ovarian regulators, the gonadotrophins (FSH and LH). This may be because fish meal indirectly affects the hypothalamus or the pituitary gland or directly sensitizes the follicular receptors to the available gonadotrophins. Similar results have also been reported by Petteri (2002). Adjene and Agoreyo (2003) reported halofantrine hydrochloride to cause retardation in growth, reduction in size of follicle, increase in cytoplasmic vacuolization and absence of corpora lutea. The formation of corpus luteum is a direct continuation of pre-ovulatory follicular development (Shivalingappa et al., 2002). In rabbit and many species estradiol is the main leuteotrophic hormone. Prolactin, FSH and LH contribute to the leuteotrophic complex as they enhance estrogen secretion by promoting the growth of large follicles (Nallbandove 1973, Trabace, 2011 and Solad, 1966). In the present study the decrease in the number of corpora lutea observed in rats treated with higher concentration of fish meal in both generations indicates that heavy metals inhibit the conversion of pre-ovulatory follicles into corpora lutea arresting ovulation. Similar results have been reported by Solomon et al., (2010).

Krajicakava et al., (2002) have reported secretory cells to have lesser number of secretory granules in the ampullary part of the goat exposed to pollutants. Barbara and Richard (1996) have reported dense cilia during estrous phase in the ampulla of rat oviduct. In the present study, the oviduct showed an increase in the number of ciliated cells with dense cilia and decrease in the number of non ciliated cells, thick mucosal layer, elongated mucosal folds projecting towards the narrow lumen in rats fed with different ratios of fish meal (1:1, 1:2 and 1:3) in both generations. These results were more prominent in F2 rats than
F1 generation rats. The above results may be due to heavy metal toxicity which causes a decrease in hormonal level in the serum, increase in the number of cilia that inhibits the movement of ova from oviduct to uterus. Similar results have also been reported by Asuman et al., (2010) in overectamized rabbits.

Uterus is a dynamic reproductive organ that undergoes cyclic changes in response to ovarian steroid hormone (Burroughs et al., 2000). Uterus of the control rats showed healthy endometrium with rounded uterine glands. In rats fed with 1:1, 1:2 and 1:3 ratio of fish meal there is increase in size of the lumen and decrease in thickness of the endometrium. Stromal cells are observed to degenerate and the endometrial glands to undergo hypotrophy. In rats fed with only fish meal there was a marked decrease in the height of the luminal epithelium with malformation of lining cells and degenerated nuclei. These results were more pronounced in F2 generation rats ( \textit{p}<0.05). The changes observed in the present study may be due to a significant decrease in progesterone level caused by heavy metal toxicity. The compound of hormonal values usually disturbs the hormonal milieu in uterus and provokes an infertility effect (Viay kumar et al., 2004). Pal (1990) reported rats treated with \textit{sesbania sesban} seeds (250 and 400 mg for 30 days) to cause a significant reduction in endometrial height, atrophy of the endometrial gland and poor vascularity. Mendoza-rodriguez et al., (2003) are of the opinion that a decrease in progesterone level causes decrease in glandular and luminal epithelial proliferation and increased apoptosis. Our results are in agreement with the previous author. Similar results have been reported by Seidlova-vuttke et al., 2003. Sheeja et al., (2009) reported the extract of leaves of \textit{plumbago rosea} to cause dialation of the endometrial
glands in treated rats. Okoko (2010) and Gbotolorul et al., (2008) have observed methanolic extract of *Abrus precatorius* seeds to cause reversible alteration in the estrous cycle pattern and also completely block ovulation in Sprague-Dawley rats.

Heavy metals alter properties of hemoglobin by decreasing their affinity towards oxygen binding capacity rendering RBC more fragile and permeable (Witeska and Kosciuk, 2003; Karuppasamy, 2000). In the present study, a gradual decrease in Hb, RBC, WBC, MCV, PCV, MCH, MCHC and platelets was noticed in rats fed with 1:1 and 1:2 ratios of fish meal during F1 and F2 generation. A significant decrease (p<0.05) in Hb, RBC, WBC, MCV, PCV, MCH, MCHC and platelets was noticed in rats fed with higher ratios of fish meal(1:3 and only fish meal) in both F1 and F2 generations. Reduction in Hb, RBC and PCV may be due to metal toxicant in the fish meal that may be destroying RBC directly or indirectly and thereby lowering haemoglobin concentration. The reduction of WBC caused leucocytosis which is directly proportional to severity of stress condition. Reduction in MCV and MCH is an indicator of hypochromic microcytic anemia a consequence of hypoxia or shrunken RBC. Similar hematological abnormalities have also been observed in fish exposed to mercuric chloride (Shakoori et al., 1991). Reduction in MCHC was observed in experimental groups indicating decrease in hemoglobin synthesis thereby lowering oxygen carrying capacity by the animal. Reduction in platelet count may be due to the presence of hyper coaguable substances in the fish meal diet which increases risk of thrombosis. Similar findings have also been reported in rats subjected to chronic cadmium toxicity (Mehtap Koçak and Ethem Akçıl, 2000). In rats fed with higher ratios of fish meal a drastic reduction in differential white blood
cell counts of neutrophils, eosinophils and basophils was observed. Monophil decrease was however not significant. This result may be due to leucopenia and lymphopenia, which leads to lowering of resistance to infectious agents. Similar results have also been reported by Hardikar and Gokhale (2002) in sewage fed fish.

Neff, (1985) reported heavy metals and pesticides to cause depletion in protein content in rats which according to them may be due to defective protein synthesis or energy diversification to meet the impending energy demand when the animal is under toxic stress. In the present study a significant decrease in the amount of protein and glycogen content was noticed in the ovary and uterus of rats fed with higher ratios (1.3 and only fish meal) of fish meal during F1 and F2 generation. These results were more significant (p<0.05) in rats fed with 1.3 and only fish meal. Decrease of protein and glycogen content in ovary and uterus might be due to interaction of heavy metals which alter the physiology of organisms by affecting important aspects of cellular metabolism. Our results are in agreement with findings of Janardhan Reddy and Sisodia (1990). The low protein content in the ovary indicates retarded ovarian growth as FSH is essential for protein synthesis in gonads (Means, 1975). Further, decreased level of glycogen in ovary and uterus may be due to low ovarian steroidogenesis, attributed to low availability of pituitary gonadotrophins. Similar results have also been obtained by Somanath Reddy Patil (1998). Fluctuations of glycogen in the female reproductive tract are correlated with the oestrous cycle and changes in steroid secretions by the ovary. Consequently, glycogen acts as a critical biomarker in female reproductive tract in normal and disease conditions. Cholesterol acts as a precursor molecule during steroidogenesis in the ovary. The present study
revealed a gradual decrease in ovarian cholesterol and gradual increase in uterine cholesterol in rats treated with different ratios of fish meal (1:1 and 1.2). A significant decrease (p<0.05) in ovarian cholesterol and increase in uterine cholesterol (p<0.05) was noticed in rats fed with 1:3 and only fish meal during F1 and F2 generation. Decrease in ovarian cholesterol resulted in degeneration of follicles in the ovary which causes reduction in hormone synthesis. Increase in uterine cholesterol content may be due to necrotic changes and reduction in the level of circulating hormones which contribute to altered physiology of the reproductive system in turn affecting steroid metabolism. Smith et al., (2005) have reported increasing cholesterol content in the uterus to have deleterious effect on uterine contractile activity. Increase in cholesterol indicates non availability of pituitary gonadotrophins for steroidogenesis.

Fertility is generally considered to be an insensitive index of oogenesis in rats. Numerous studies have shown that significant decrease in ova number can be associated with fertility in rodents (Blazak et al., 1985). Wiersma et al., 1986, have reported lead and cadmium to affect the reproductive performance in female animals in terms of, reproductive organ, endocrine system and embryonic development. In the present study rats fed with higher concentration of fish meal (1:3 and only fish meal) in both F1 and F2 generations showed reduced fertility index. The decline in fertility rate in the present study may be due to impaired ovarian function resulting from impaired action of the gonadotropin and also an increase in gonadal steroidal inhibition. Mogra et al., (2009) reported lead exposure to have adverse effects on gestation and development during the first trimester. Results obtained in the present study revealed an increase in gestation length in rats fed with higher ratios of fish meal during both F1 and F2
generations. This result is due to hormonal level alterations, fetal growth and also associated dystocia.

Implantation in rats depends on completion of basic sequence of events occurring both in fertilized eggs and endometrium. The endometrium needs a forty eight hour period of progesterone preparation and the presence of estrogen at the end leading to formation of high sensitive decidualized endometrium (Sharanabasappa and Saraswathi patel, 2002). The imbalance in this hormone leads to failure of implantation (Ghosh et al., 1997a). In the present study rats fed with different ratios of fish meal showed decrease in number of implantation. With increasing ratio of fish meal this result was more prominent in F2 generation. These findings may be due to metal toxicants in the fish meal that causes imbalance in the hormones for endometrial preparation or failure of egg and endometrial interaction. Ahefez et al., (1976) have also reported loss of implantation sites to be caused by administration of phenolphathalein. Rats exposed to lead show post implantation loss which may be because the metal binds with the critical membrane sites or intracellular ligands including proteins and nucleic acids which triggers inhibition of development loss in pregnant females (Jackquet 1975). Ragini Sharma et al (2012) showed reduced number of pregnancy, small litter size and decreased body weight in animals treated with Lead. Our results are in accordance with previous authors.

Rats fed with higher ratios of fish meal revealed decrease in number of corpora lutea in both generations. This result may be due to reduction in the number of ovulation, fertilization and embryo death caused by metal toxicity. Cummins et al., (2000) have reported decrease in serum progesterone to cause decrease in implantation and increase in resorption in atrazine treated rats. In the present study significant increase in
resorption was observed in rats treated with higher ratio of fish meal (1:3) and only fish meal during F1 and F2 generation. Increase in implantation loss may be due to suppression of PRL levels which impairs corpora lutea maturation. Helal and Dakdoky, (2006) noticed increased frequency of resorption of dead fetus at birth and external abnormalities per litter in the females of sodium treated groups. Mona et al., (2000) observed less number of viable foetuses, lowered implantation site and higher number of resorptions with increased and relative weights of ovaries in rats exposed to fluoride.

Gebrie et al., (2005) have reported that methanolic root extract of Rumex steudelii caused a significant reduction in the number of litters and a significant decrease in the number of implantation sites. In the present study F2 generation rats fed with higher ratios of fish meal showed significant (p<0.05) reduction in live birth which may be due to heavy metal toxicity that produces an unfavorable endometrium for implantation of the fertilized ovum. High doses of cadmium intake may result in abortions, low weight at birth and congenital abnormalities (Baranski, 1986 and Soukupova and Dostal, 1991). Lead during gestation causes decrease in body weight of the mother, foeti and live foeti. In the present study the weight of the pups was observed to decrease in rats fed with higher ratios (1:3 and only fishmeal) of fish meal in both the generations. The observed retardation of fetal growth may be due to inhibition of fetal hemoglobin synthesis as heavy metal intoxication causes anemia or an action on placental blood flow. These results are in accordance with Fatma and Fakhry, 1992; Randa and Ibrahim 2006; Gerber and Maes, 1978. In the present study significant reduction (p<0.05) in the number of young ones was observed in rats fed with only fish meal during F1 and F2 generation.
compared with other ratios and control. This may be because of miscarriages due to weak reproductive organs caused my metal toxicity. Similar results have been reported in rats subjected to cadmium toxicity by Eteng et al., 2008.

In the present study the Pups weight (0 and 21 days) of F1 and F2 generations rats fed with ratios of 1:3 and only fish meal showed reduction in birth weight in F1 and F2 generation compared with control. This result is attributed to the carryover of reproductive malformation trait of the parent rat caused by toxic distortion of the female reproductive organs by heavy metal toxicity. This result is in accordance with Popharm and Webster, (1979). In some investigations birth weight was reduced (Galler, 1991; Vickers et al., 2001), in others unchanged (Langley-Evans et al., 1994; Langley-Evans et al., 2000; Brawley et al., 2003; Lamireau et al., 2002 ) and in some even increased (Langley-Evans et al., 1996). This discrepancy may be due to factors that may alter response of the mother and fetus to nutrient restrictions such as strain, maternal age and differences in the diet (Franco et al., 2002; Gangula et al., 2005). Similar results have been obtained by dietary restriction studies carried by Satish Kumar (2009).