DISCUSSIONS
The survey of the existing literature reveals that a few frog species have been used to study: the toxic actions of heavy metals such as iron, cadmium, nickel, copper, cobalt, aluminium, magnesium, lead and zinc; pesticide actions; actions of pH shifts of water bodies on the fertilization; development and metabolism; action of mine drainage; teratogenic effects of different contaminants; effect of water quality on mortality rate; ion up takes by the respiratory surfaces; effects of contaminants on growth.

Many researchers have used African frog *Xenopus laevis* to study the actions of different pesticides and heavy metals for FETAX (Frog Embryo Tetratogenic Assay: *Xenopus*) studies. A few have used other amphibians like salamanders and toad species for environmental monitoring studies. Now it is known that environmental deterioration is causing wide spread decline of amphibian species as these have been proved to be very sensitive to the slightest change in the environment. As the frogs are mostly found in the wet lands and aquatic bodies they are more susceptible to even subtle changes in the habitat. Especially the frog’s developmental stages have to live for a longer duration in water bodies thereby making them most vulnerable to the contaminants entering into the aquatic systems/habitats. Besides being constantly in water during development they are likely to get exposed to different contaminants through food. Because of the vulnerability to subtle to moderate changes in the environment the Amphibian Decline Task Force has been established to study the decline of amphibian population and to save the endangered amphibian species.
Goa has been experiencing the degradation of environment, especially due to extensive mining activities and stock piling of iron ore rejects. These stockpiles become the constant source of mining effluents which contaminate the receiving water bodies. The water quality of these mining effluents has been already assessed (Desai, 1990; Bandiwdekar and Desai, 1998). The present study confirms the deteriorating actions of these mining effluents originating from the iron ore stockpiles. The heavy metal analysis of these mining effluents generated through simulated rainfalls clearly show the presence of heavy metals like barium (0.65 mg/l), cadmium (0.71 mg/l), cobalt (0.022 mg/l), chromium (0.037 mg/l), copper (0.014 mg/l), iron (1.91 mg/l), magnesium (0.21 mg/l), manganese (0.85 mg/l), nickel (0.11 mg/l), lead (0.005 mg/l), strontium (0.01 mg/l) and zinc (0.081 mg/l).

Besides this the physico-chemical characteristics show that mining effluents have high load of total solids, total phosphorous, organic phosphorous, sulphates and nitrates. The large quantity of such mining effluents if mixed with natural water body would definitely pollute it and pose danger to the aquatic forms. Similar observations on the mining effluents have been reported by Desai (1990).

There are reports of toxic actions of heavy metals on frog eggs (Birge et al., 1979; Kaplan et al., 1967; Berisha and Rozhaja, 1989; Hopfer et al., 1991) with reference to mortality, teratogenesis, hatching, early larval developments. But there is hardly any report of toxic actions of mining effluents or actions of heavy metal mixtures on the development of tadpoles and deformities, histopathological changes, haematological changes and biochemical alterations with reference to total proteins, glycogen, AP, NSE, AlP, electrolyte
concentrations in different organs like skin, liver, kidney, intestine and muscles of tadpoles of any frog species.

A steady decline of amphibian species and specially estuarine fauna is observed by Parulekar et al., (1986). The present investigation clearly indicates the contribution of mining effluents in the decline of frog species; especially how the tadpoles are affected by mining contaminants resulting in the development of deformities, histopathological lesions of skin, intestine, liver and kidney as well as the changes in a few biochemical profiles of these organs.

It is clear from the present study that mining effluents ranging from 0.1 to 30.0 % reduce the amplexuses of adult frogs and the spawning of female frogs. It is also seen that mining effluents do not produce teratogenic changes in the embryo. It appears that all the stages of *R. tigerina* are equally vulnerable to 30 % ME as LC 50 remains unchanged. The study of NOEC (no observable effective concentrations), LOEC (lowest observable effective concentration) indicates that ME concentrations from 0.001 to 0.008 % are relatively safe while at 0.009 % concentrations of ME, the tadpoles get slightly irritated as indicated by excessive wriggling. But ME concentrations ranging from 0.01 to 30.0 % do not interfere into the embryonic development leading to the formation of limb bud stages, and also they do not develop deformities in the limb bud stages from I to IV.

**DEFORMITIES IN THE TADPOLES:**

0.01 to 30.0 % ME induced deformities in the various tadpoles' stages and these deformities are kinking of tail, loosening of gut coil, flexure of the notochord and an abnormality like stunted growth.

**STAGE V:**
The present study indicates that 5.0 % ME is comparatively least effecting in producing deformities, while 30.0 % ME is more effective as it produced the maximum of 51 % deformities and the number of deformities depend on the concentration and exposure period.

STAGE VIII:

The deformities produced in tadpoles of stages VI to VIII are similar with respect to the nature and percentages of deformities which indicates that ME is equally effective at this stages. Like stage V thirty percent ME is most effective in producing deformities. The number of deformities produced depend upon ME concentration and exposure period but 0.01 % ME is not effective up to the end of forty eight hours, 0.1 % ME up to twenty four hours, 1.0 % ME up to twelve hours while 5.0 % ME is not effective up to six hours.

STAGE XIV:

ME produces identical pattern of deformities in the tadpoles of stages from IX to XIV indicating equal effectiveness. 0.01 % ME is not effective up to the end of seventy two hours, while 0.1 % ME is not effective up to the end of twelve hours. ME concentrations like 1.0, 5.0 and 10.0 % are not effective up to the end of six hours and 30.0 % ME is the most effective concentration. It seems that percentages of deformities developed in the tadpoles are not exclusively exposure period or ME concentration dependent.

STAGE XVIII and XXII:

As seen in previous stages, ME is equally effective in producing deformities in tadpoles of stages XV to XVIII and stages XIX to XXV. The present study shows that 0.01 % ME is not effective upto the end of forty eight hours and
rest of the concentrations of ME are not effective upto the end of twenty four
hours indicating the resistance to deformities of these stages at these exposure
periods.

The present study establishes the intolerance of tadpoles to mining
effluents of moderate to higher concentrations and also to very low
concentrations if exposed chronically. Birge et al., (1979) reported the most toxic
metals tested with the toad (Gastrophryne carolinensis) were mercury, silver, zinc,
chromium, lead, cadmium, copper and arsenic and showed that acute LC50s for
these heavy metals ranged from 1 to 100 ppb for the toad which indicates greater
sensitivity relative to Xenopus laevis. Barinaga (1990) has summarized the
mounting evidence that frog population are rapidly declining on a world-wide
basis, possibly caused by acid rain, environmental toxins, climatic change and
nickel contamination of surface waters could be contributory factor.

In the present investigation mining effluents are inducing deformities and
abnormalities like kinking, malrotation/loosening of intestine, flexing and
stunted growth. As the mining effluents contain heavy metals like barium,
cadmium, cobalt, chromium, copper, iron, magnesium, manganese, nickel, lead,
strontium and zinc it is possible that the deformities and abnormalities produced
in different tadpoles of stages could be due to the actions of heavy metals
depending upon their absorption during the development of tadpoles by
different tissues. Similarly have reported heavy metal induced deformities in
some frog species (Miller and Landesman, 1978; Abbasi and Soni, 1984; Dawson
et al., 1988; Berisha and Rozha, 1989; Plowman et al., 1991; Hopffer et al., 1991;
Sunderman et al., 1991; Luo et al., 1993a,b; Read and Taylor, 1994; Horne and
Dunson, 1995). All the heavy metals might not have worked synergistically as magnesium and zinc could have decreased the severity, as they are known to antagonize the action of chromium and nickel (Herkovits and Perez-Coll, 1990; Luo et al., 1993).

In the present work, the physico-chemical analysis of mining effluents has revealed that ME bears acidic pH and lower hardness level. These conditions may have aggravated the toxic actions of heavy metals as suggested by Horne and Dunson (1995). It needs further investigation to find out which of the heavy metals have been absorbed and whether magnesium and zinc have antagonised the action of some heavy metals. Gosner and Black (1957), Pough (1976), Pough and Wilson (1977), Schlichter (1981) are of the opinion that acidification of water alone can impair larval developments.

**HISTOLOGICAL ALTERATIONS:**

The histological studies of skin, intestine, liver and kidney of different larval stages of *Rana tigerina* exposed to different concentrations of mining effluents (0.01 to 30.0 %) for exposure periods ranging between 6 hours to one hundred and twenty hours revealed histopathological alterations in the tissue architecture indicating deleterious actions of mining effluents. The nature and acuteness of damage of these tissues depend upon the strength of the mining effluents and the length of the exposure period.

**HISTOPATHOLOGY OF SKIN:**

The sections of the skin of tadpoles exposed to various concentrations of mining effluents for different time periods when stained with haematoxylin and eosin revealed the distortion of normal architecture of the skin. ME induces
disruption of the epidermal and dermal region. Mining effluents induce in a concentration and exposure period dependent manner necrosis of sensory papillae, vertical muscle, stratum spongiosum, stratum compactum and lesions of stratum Malpighii and stratum corneum. The low concentration of ME like 0.01 and 0.1 do not have any effect up to the period less than forty eight hours for tadpoles of stages V and VIII which indicates that the tadpoles skins at early stages would escape damage while those of stage XIV could be escaping damage up to twelve hours and these up to stage XVIII to XXV could escape damage of the skin up to the end of twenty four hours when exposed to 0.01 % ME. The present study indicates that the low concentrations of ME could be toxic to the skin after twenty four to forty eight hours exposure while those above 0.1 % could have pronounced damaging effect. The necrosis of sensory papillae could lead to the loss of tactile sensations. The lesions of stratum Malpighii could obstruct formation of new cells which may impair normal regeneration or repair of the skin layers and eventually formation of stratum corneum which could open the gates for infectious germs. The lesions of stratum spongiosum vis-à-vis of vertical muscle would disturb the basic architecture of skin leading to the loosening of tadpole skin which normally is closely fitted to the body of tadpoles. The loosening of tadpole skin could impair, disturb the dermal blood supply, thereby making the whole affected portion of the skin dead. Physiologically the tadpole skin does act as an endocrine organ (Wassersug, 1997). Anuran skin contains a vast pharmacopeia of neuroactive and vasoactive peptides (Erspamer, 1994). As tadpole skin is an endocrine organ, it could release its hormonal products into the circulatory system in direct response to external influences on
the skin. The most obvious of these influences is water itself, which is absorbed through the skin. A great many peptides (tachykinins, bradykinins) bearing vascular and renal effects could act through feed back loops to regulate overall hydration. One possibility is that these compounds are washed from the skin into anurans as water comes in through skin itself. It is also known that during metamorphosis into a froglet which hops out of water, the skin gets irreversibly dried out (Wassersug, 1997). The lesions of the skin could lead to impairments of endocrine functions of skin leading to the disturbed vascular and renal functions. Besides this the tadpole skin is known to regulate ionic balance and is involved in passive and active transport of ions (Wassersug, 1997). The lesions, necrosis of skin could even lead to the disturbances of ion transports. Cadmium effects epithelial cells by inhibiting active ion transport by combining with sulfhydryl groups in the cell membrane and decreasing membrane potential (Kanno et al., 1978; Arita et al., 1979; Arhem, 1989; Takada and Hayashi, 1981a, b). Binding of lead in amphibian skin is reported by Ireland et al., (1979) and they attributed this binding to the affinity of the melanin to the metals. Binding of lead to the sulfhydryl groups and disruption of sulphur bridges is reported by Celentano et al., (1979); Kanno et al., (1978) reported zinc combining with sulfhydryl groups in the amphibian cell membranes and have shown the tendency of zinc to form complexes with certain ligands. Landé and Guttman (1973) reported iron, zinc and copper toxicity to tadpoles and Arhem (1980) reported nickel, zinc, copper and cobalt binding with sulfhydryl groups in cell membrane. Metals are not only toxic to amphibians but are also readily accumulated in body tissues and can be concentrated as they are passed up the food web (Byrne et al., 1975; Baudo, 1976;
Browne and Dumont, 1779). Metals can be concentrated in amphibian tissues many times environmental levels and in some instances the metals are retained for long period of time (Ireland, 1977; Browne and Dumont, 1779; Canton and Sloof, 1982). As many of the above cited heavy metals are present in the mining effluents, it is possible that the heavy metals are absorbed by the skin and may have bound to the sulphydryl groups to get accumulated. The degree of absorption and accumulation could decide the severity of the lesions caused to the tadpole skin. Such tadpoles would fail to metamorphose normally.

**Histopathology of Intestine:**

The histopathological studies of intestine of tadpole stages from one to twenty two exposed to mining effluents ranging from 0.1 % to 30.0 % revealed that ME induce minor histopathological changes of the intestine of early tadpole stages like one to four in the form of swelling of villi of cells. The number of cells showing early necrotic changes depend upon the concentrations of ME and exposure period. The histopathological study of intestines of tadpoles of stages five to twenty two indicate ME induce necrotic lesions. The present study clearly shows that ME induced necrosis of villi cells, goblet cells, lesions of villi, lesions in the serosal layers, disruptions of lamina propria, muscularis mucosa as well as loss of villi, sloughing off of cells, ME induce these changes of concentrations and exposure period dependent manner. Every concentration of ME at every exposure period has induced lesions of villi, especially low concentrations of ME induce patchy necrosis of the intestine along with the lesions of villi. Only tadpoles of stage V escape significant damage up to twenty four hours but do show signs of beginning of necrosis as evidence by swelling of cells. The loss or
lesions, and necrosis of villi cells would disturb the normal functioning of villi i.e. absorption of solutes and electrolytes from the digested food along with the disturbances of process of digestion. The loss of cells of villi, their swelling would definitely disturb the secretory activities of intestine leading to the decrease in the secretions of intestinal hormones and enzymes. The necrosis of goblet cells would disturb the mucous secretions and the lesions of the muscularis mucosa would disturb the intestinal motility. If intestinal motility is disturbed the digestion and movement of the chyme in the intestine is likely to hamper. Besides this a putative hormone villi-kinin secreted by the mucosal layer is responsible for the movement of villi. If mucosal layer is disturbed/damaged it could lead to the loss or decrement of the motility of the villi. The lesions of the lamina propria would result into the loss of core of the villus, loss of lymphocytes, loose connective tissue and isolated smooth cells. These histopathological changes of villa in general would lead to serious disturbances in the digestion and absorption of food by the tadpoles which may affect the normal development and metamorphosis in general.

The intestinal histopathological changes could be attributed to the different heavy metals present in the mining effluents. The tadpoles take in water through the mouth and pass it over the internal gills for respiration. During this process a little water/mining effluent is likely to pass into the gut. Besides this the intake of food could also promote entry of mining effluents into the gut. The heavy metals from this could be absorbed by the intestine and depending upon the absorption and accumulation of heavy metals the necrosis and lesions of the intestinal parts get effected. It is also possible that the heavy metals like nickel
could induce damage to the intestine by inducing oxygen free radical reactions (Sunderman, 1985; 1987; Hopfer et al., 1991). The present study also reveals the involvement of lysosomal enzymes like AP and NSE as these enzymes' activities have been found to elevate during the bioassays of enzymes of intestine. Therefore the activities of lysosomes by the heavy metals or heavy metals associated reactions could lead to necrosis/lesions of intestinal villi or lamina propria, muscularis mucosa and there is a possibility of activation of the process of apoptosis by heavy metals which could promote death of intestinal cells. There are reports of cadmium induced inhibition of active transport by epithelial cells by combining with sulfhydryl groups in the cell membranes and decreasing membrane potential (Kanno et al., 1978, Takada and Hayashi, 1981a,b). Also lead is known to bind to sulfhydryl groups and disruption of sulphur bridges leading to the change in structure of membrane proteins leading to changes of ionic channels (Celentano et al., 1979). The acute changes of the proteins of membranes could lead to lesions of membrane which eventually would promote loss of cells. The acuity of protein alterations and membrane lesions, cell necrosis would depend upon the quantity of heavy metals entering the intestine, its absorption and accumulation.

**Histopathology of Liver:**

The liver of the tadpoles of stages I to IV exposed to mining effluents ranging from 0.01 to 30.0 % undergo minor changes like paling of liver or discoloration of liver and the liver sections show the swelling of a few hepatocytes. The number of hepatocytes getting affected depend upon the ME concentration and exposure period. However, the livers of the tadpoles of stages
V to XXII undergo necrotic changes under the influence of mining effluents. Mining effluents induce histopathological changes like widening of sinusoids, increase of the number of Kupffer cells, necrosis of endothelial cells, decrease in pigmentation/pigment granules, necrosis of hepatocytes, pycnosis of nuclei of hepatocytes and accumulation of debris in the sinusoids. The degree of necrosis and acuity of the changes depend upon the concentrations of mining effluents and the length of exposure of tadpoles to ME.

The enlargement of sinusoids could be due to the stepping up of blood supply to the interlobular vein which pours it into the sinusoids and enlargement of sinusoids could subsequently reduce the blood pressure. The first phase of enlargement indicates excessive blood supply which could be the result of over accumulation and absorption of heavy metals which are taken to the liver for detoxification. Liver is known to be the seat of detoxification, especially the centre for the synthesis of metalloproteins which could bind to the heavy metals brought into the liver for detoxification. For these activities more blood need to be supplied to the liver. The over accumulation of heavy metals in the liver could result in the necrosis of hepatocytes which may be due to the formation of oxygen free radical reactions as suggested by Hopfer et al., (1991) or due to the activation of lysosomes. In the present investigation increase of the AP and NSE of liver is noted. The increases in these enzymes are known to be associated with necrosis of cells and tissues (Dingle and Fell, 1969). Kupffer cells are phagocytic, therefore increase of the number of Kupffer cells could be for phagocytosis of debris accumulated in the sinusoids due to the loss, necrosis of hepatocytes as well as endothelial cells and blood cells. Endothelial cells are known for pinocytosis and
accumulation of heavy metals in them could lead to their necrosis. The decrease in the pigmentation of liver could be due to decrease in number of hepatocytes or decrease in the synthesis of pigments by the hepatocytes as they are known to synthesize bile pigments like bilirubin. Discoloration of the liver of *Necturus* reported by Dawson (1933), in *R. catesbeiana* by Barrett, (1947) and in *R. pipiens* by Kaplan et al., (1967). These authors have attributed this to accumulation and metabolism of lead as well as phagocytosis of red blood cells. As mining effluents has lead in it along with other metals, it could be assumed that the discoloration of liver of tadpoles of stages I to IV and of higher stages is due to the lead or other heavy metal metabolism and also due to phagocytosis of blood cells by Kupffer cells when their number rises significantly. The damage/necrosis of hepatocytes under the influence of heavy metals from the mining effluents may lead to impairment of fat digestion since bile acids produced by hepatocytes act as cofactors in fat break down (Buchan, 1989). Also damage to the liver could lead to decrease in the glycogenesis as well as glycogenolysis. In short metabolism of the tadpoles would be affected under the influence of mining effluents leading to disturbances in the metamorphosis of tadpoles.

**Histopathology of Kidney:**

The routine haematoxylin-eosin stained sections of the kidneys taken at the end of six, twelve, twenty four, forty eight, seventy two, ninety six and one hundred and twenty hours after exposure of tadpoles of stage V to XXII to 0.01, 0.1, 1.0, 5.0, 10.0 and 30.0 % mining effluents (ME) exhibited ME concentration dependent and up to a large extent exposure period dependent necrosis of glomeruli, proximal, distal and collecting tubules. The kidney of tadpoles of
stage I to IV some how appear to escape severe damage but the ME induce early necrotic changes of them as evidence by swelling of glomeruli, proximal and distal convoluted tubule. But the tadpoles of higher stages do not get affected by the mining effluents right from the end of six hours.

The tadpoles exposed to a lower concentration of ME (0.01 %) show the renal damage as early as six hours in the form of widening of interstitium in all stages and swelling or necrosis of glomeruli, widening of Bowman’s space in the higher stages of tadpoles up to the end of one hundred and twenty hours. Thus, in a large water body if mining effluents mix to such an extent that the water body possesses/reaches the heavy metal concentrations equivalent to that found in 0.01 % ME or even little less the renal tissues of the developing tadpoles of frog getting exposed for a period of few hours could get progressively damaged. On the other hand the heavy metal concentrations of the water body keeps on rising as the mining effluents keep on mixing in it during monsoon, especially in the areas adjacent or away from the mines. The tadpoles metamorphosing in them would get affected adversely.

From the present investigation it appears that the glomerular changes of general are beginning with glomerular swelling and then progress towards distortion which include diruption of glomerular walls, vacuolisation, release of glomerular exudents into the Bowman’s space, breaking of capillaries, exudation of glomerulus resulting in emptying of Bowman’s capsule. The alteration in Bowman’s capsule are like the widening of Bowman’s space and disruption of Bowman’s inner and outer walls. The number of glomeruli affected depend upon ME concentration and exposure period. In the stage where glomeruli are not
developed, the damage to the renal tubules is induced by ME. As the concentration of ME increases, more and more glomeruli become necrotic.

The necrotic changes of the proximal and distal convoluted tubules are identical under the influence of various concentrations of mining effluents. The early necrotic changes of the proximal and distal tubules include swelling of tubule cells seen as early as twelve to twenty-four hours. The prominent features of necrosis of proximal and distal tubules are: swelling of tubule cells, disruption of tubules, vacuolisation of tubule cells, loss of brush, luminal border, loss of basal membranes, pycnosis of nuclei of tubule cells, widening of interstitium and accumulation of debris in interstitium of the cytoplasmic or nuclear origin.

Similarly different concentrations of ME induce necrosis of collecting tubules depending upon the concentration of ME and exposure period. Most of the necrotic changes of the collecting tubules are similar to those observed in the proximal and distal tubules. The causes of glomerular, proximal, distal and collecting tubules' necrosis could be the toxicity caused by the accumulation of heavy metals in the kidney. The tadpoles actively absorb water through skin (Wassersug, 1997) and the water coming through food into the intestine would bring in the heavy metals present in the water into the body of tadpoles. In the present case the mining effluents mixed in water could find their way through above mentioned routes into the tadpole body. The tadpoles may try to eliminate some of the heavy metals through kidney and in the process may accumulate some of the heavy metals which may cause renal necrosis depending upon the accumulation of heavy metals and the metals load of the kidney and body in general. There is hardly any report on the renal lesions caused by the heavy
metals in the tadpoles of frogs. However Bandiwdekar (1996) has studied the deleterious effects of mining effluents on the kidney of ducks and has attributed these renal necrotic changes to the accumulation of heavy metals in the kidney and blood as well. Similarly Nicholson and Osborn (1983) hold high tissue levels of cadmium and mercury responsible for kidney lesions of pelagic sea birds while Muirhead and Furness (1988) correlate kidney lesions of sea birds to the concentrations of mercury, cadmium, copper and zinc. Besides these, there are several reports on the heavy metal induced lesions in the fish kidney (Sobodash, 1974; Rosenthal and Sperling, 1974; Benoit et al., 1976; Wong et al., 1977; Zit Ko and Carson, 1977; Kumar and Pant, 1981; Singh, 1983; Mukhopadhyay and Konar, 1985; Sharma and Sharma, 1995).

In the present work kidney necrosis of tadpoles could be due to the accumulation of heavy metals as mentioned earlier but it is difficult to correlate any single heavy metal to the renal damage and hence the combination of the various metals could be considered as factors responsible for renal damage, though up to certain extent a limited contribution of a specific metal’s highest accumulation at specific time interval could also be admitted. Even, accumulation of lead or cadmium could also induce nephrotoxicity as suggested by some researchers. The renal necrosis may partly be also due to the altered blood supply as proposed by Tomera et al., (1991).

HAEMATOLOGICAL CHANGES:

Haemoglobin:

The present study clearly shows that haemoglobin concentrations of the tadpoles of stages V to XIV increase under the influence of mining effluents in the
range of 2.59 to 2.95 grams per decilitre, especially the lower concentrations of ME produce more increase of haemoglobin concentration at the end of six hours and as the concentrations of ME increase the haemoglobin concentration drops but it remains quite above that observed in the controls till the end of seventy two hours. Thus, ME promotes inverse relationship of ME concentration and haemoglobin elevation. Elevations of haemoglobin concentrations at the end of one twenty hours are not so pronounced. In tadpoles of stage XIV, ME concentrations like 10.0 and 30.0 percent induce decrease in haemoglobin level from the end of over ninety six to one hundred and twenty hours. This typical pattern of alterations of haemoglobin concentrations of the blood of tadpoles of stages V to XIV under the influence of ME appears to be exclusively due to the identical pattern of changes of erythrocyte counts/numbers under the influence of mining effluents. Thus, the present work indicates that the elevations and the declines of haemoglobin levels in the blood of tadpoles are due to the elevations and declines in the erythropoiesis vis-à-vis erythrocyte counts under the influence of mining effluents.

The mining effluents induce nearly concentration and exposure period dependent decline in the haemoglobin levels of tadpoles of stages XV to XVIII and the haemoglobin level falls below those exhibited by the control tadpoles. The mining effluents promote a perfect concentration and exposure period dependent decline in the haemoglobin levels of the tadpoles of stages XIX to XXII. The decline of haemoglobin in these stages of tadpoles is pronounced and progressive especially after twenty four hours exposure to ME.
The changes of the haemoglobin levels of the blood of tadpoles of stages XV to XXII under the influence of ME are significantly parallel to those changes observed in the erythrocyte counts of those tadpoles of stages exposed to mining effluents. There is hardly any report on the heavy metal induced alterations in the haemoglobin levels of tadpoles. However, Mathew et al., (1997) have reported lead nitrate induced reduction of haemoglobin contents of fish, *Cyprinus carpio*. There are also other reports of heavy metals induced reduction of Hb contents of fishes (Rai and Qayyam, 1984; Singh and Bhati, 1994). Therefore, the decline in haemoglobin of the late stages of the tadpoles could be considered due to the toxic actions of heavy metals from the mining effluents as observed in fishes. Dhanekar et al., (1985) reported increase of the haemoglobin contents of juvenile fishes (*Heteropneustes fossilis* and *Channa punctatus*) due to zinc toxicity. In the present investigation elevations in the haemoglobin levels of early tadpole stages (V to XIV) is seen. These elevations also run parallel to elevations of erythrocyte counts indicating direct relationship between the haemoglobin contents and erythrocyte number. Hence, it can safely be assumed that some heavy metals like zinc from ME with a possibility of involvement of other materials, might be influencing haemoglobin synthesis as well as erythropoiesis in the tadpoles.

**ALTERATIONS OF RBC COUNT:**

The present investigation reveals that the control tadpoles exhibit steady increase of the RBC count from the end of six hours to the end of one twenty hours but the tadpoles of stage XXII to XXV show sharp increase of the erythrocyte count from the end of forty eight hours onwards.
Mining effluents (ME) induce increase of the RBC count of tadpoles of stages V to VIII at the end of six hours and erythrocyte number ranges between 0.87 to 2.56 millions per cubic millimeter. Lower concentrations of ME induce more increase of erythrocyte number while the higher concentrations of ME promote comparatively lesser elevations of RBC count at the end of six hours and subsequently the RBC count declines steadily up to the end of one twenty hours but it remains above that seen in control tadpoles. ME induce sharp increase of RBC counts of tadpoles of stages XIV to XVIII and the RBC count elevates to a range of 2.3 to 2.8 millions per cubic millimeter. Here also elevations of RBC counts are inversely proportional to the concentrations of mining effluents i.e. lower the concentration of ME higher is the elevation of RBC count. The RBC count is declined by ME subsequently. Ten and thirty percent concentrations of ME appear to decline RBC count at the end of one twenty hours while remaining concentrations of ME though decline the RBC count steadily, it remains above the control levels.

The present investigation reveals that mining effluents promote decline of RBC count in tadpoles of stages XVIII to XXV. ME induces concentration and exposure period dependent decline of the erythrocyte count. Higher concentrations of ME induce sharp declines of RBC counts. The increase of the RBC count i.e. erythrocyte count in the tadpoles of stages I to XIV under the influence of mining effluents indicate the stimulating actions of ME on the erythropoiesis, especially low concentrations of ME appear to stimulate erythropoiesis more effectively than the higher concentrations and subsequently more absorption and retentions of heavy metals by the tadpole tissues from ME.
could be suppressing erythropoiesis and destructions of RBCs, leading to the decline of RBC count. It is already known that erythropoiesis depends upon several factors, especially the formation of erythropoiesis-stimulating hormone (ESH) i.e. erythropoietin plays significant role in induction of erythropoiesis. There are several factors which stimulate erythropoiesis, synthesis and release. They are: hypoxia/oxygen deficiency, bleeding, vasopressin, haemolysates, corticotropins. In the present work the skin and other organs receive injury which leads to bleeding and eventually the blood cells get destroyed. The products of blood cell destruction are called lysates. Therefore the bleeding and lysates could have promoted the erythropoietin release causing enhanced erythropoiesis. The skin of frog works as an endocrine organ and contains a vast pharmacopoeia of neuroactive and vasoactive peptides (Erspamer, 1994). One of these peptides is sauvagine (Corticotropin releasing hormone) and is found in high concentration in frog/tadpole skin and Wassersug (1997) opines that any perturbations of skin (i.e. injury) may lead to its release into the blood stream. As the skin of tadpoles is found to be injured due to the exposure to mining effluents even in early stages, the first batch of sauvagine could be released in circulation which could promote release of corticotropin and under the influence of corticotropin the erythropoiesis could be stepped up (Wassersug, 1997). This might be the reason for shooting up of RBC counts in early tadpole stages and as the tadpoles keep on growing to late metamorphic stages this sauvagine mechanism might not be working effectively but it needs further investigation to throw adequate light on this aspect. There is hardly any report on heavy metal induced changes of RBC counts of tadpoles. Dhanekar et
al., (1985) reported increase of the RBC count of juveniles of fishes like *Heteropneustes fossilis* and *Channa punctatus* due to zinc toxicity. May be zinc present in the mining effluents could be contributing to the elevation of RBC counts. The higher stages of tadpoles show sharp reductions of RBC counts at the end of six hours and these reductions progress further up to the end of one twenty hours. Similarly the tadpoles stages from I to XIV show reduction of RBC count as the length of exposure periods to ME increases. Kaplan et al., (1967) reported reduction of red and white blood cells of *R. pipiens* exposed to lead nitrates. Mining effluents contain lead along with other heavy metals which could also be contributing to the reduction of RBC counts.

At present it is very difficult to say which of the heavy metal alone is influencing erythropoiesis. It is also possible that some of the metals from mining effluents are reducing the RBC count depending upon the absorption and retention/accumulation of heavy metals in the body which is also likely to depend upon the concentration of mining effluents and exposure period. Mining effluent has iron as a predominant metal and the lower concentration of ME when presented to tadpoles the iron absorbed by the skin and intestine in low concentration could elevate haemoglobin concentration and erythropoiesis to step up RBC count. Hence there could be increase of RBC count of some tadpole stages up to certain extent but it is difficult to know why the same fails in tadpole stages ranging from XIX to XXII. The reduction of total RBC count may also be due to the synergistic actions of heavy metals from mining effluents affecting erythropoiesis. Unfortunately there is hardly any work reported on the heavy metal induced alterations in the erythropoiesis of tadpoles. But the reductions of
the count of tadpoles are well in agreement with the findings of several researchers in the fishes, under the heavy metal toxicity. Banerjee and Kumari (1988) reported reduction of total RBC count in \textit{Anabas} under the influence of zinc, mercury and cadmium. Shandilya and Banerjee (1989) reported erythropenia (decrease in erythropoiesis/RBC count) in \textit{Heteropneustes} under the influence of zinc and chromium. Rao \textit{et al.}, (1990) reported decrease of total RBC count of fishes inhabiting polluted waters of Vishakhapatnam harbour. Allen (1993) reported lead induced reduction of total RBC counts in \textit{Oreochromis}. Mukherjee and Sinha (1993) showed lead induced decrease of total RBC count of \textit{Labeo}. Wepener \textit{et al.}, (1992) opined that the use of haematological methods as indicators of sublethal stress can supply valuable information concerning the physiological reactions to the changing environment. Saravanann and Natarajan (1991) argued that haematological parameter changes under metal toxication, are indicators of metallic stress. Therefore the reduction of total erythrocyte count of tadpoles under the influence of mining effluents may be considered as an indication of stress. The degree of renal necrosis would disturb the release of renal erythropoietic factor, thereby decreasing erythropoiesis (Ersler, 1975).

**ALTERATIONS OF WBC COUNT:**

The mining effluents induce concentration dependent increase of total WBC count of tadpoles of stages I to V and further they promote fluctuations at a few time intervals but elevate WBC count significantly at the end of one twenty hours. Mining effluents promote concentration dependent but steady increase of WBC count of tadpoles of stages VI to VIII but WBC count, almost levels of a six hour level at the end of twelve and twenty four hours and then steadily increases.
But mining effluents induce very sharp elevations of WBC counts of tadpoles of stages IX to XIV at the end of forty eight hours under the influence of 5.0, 10.0 and 30.0 % ME. No other concentration of ME could induce such a sharp elevation while ME concentrations from 0.01 to 5.0 % induce elevation of WBC count in the concentration dependent manner at the end of six hours and subsequently ME induced steady elevation of WBC count. But ten and thirty percent ME induced pronounced and sharp increase of WBC counts at the end of seventy two hours. WBC counts increased in a ME concentration dependent manner, in tadpoles of stages from XIX to XXII and it slightly fluctuated at some time intervals but at the end of one twenty hours raised significantly. The WBC count was very high as compared with that observed in the control tadpoles.

There is hardly any report on the actions of heavy metals or mining effluents on the leucocyte counts of tadpoles. Kaplan *et al.*, (1967) reported decline of leucocyte count of adult frogs exposed to lead nitrate. The elevations of leucocyte count (WBC counts) are well in agreement with the observations made by several researchers on the action of heavy metals in the fish leucocyte counts (Shandilya and Banerjee, 1989; Rao *et al.*, 1990; Gill and Epple, 1993; Alkahem, 1994; Allen, 1994). The fluctuation of leucocyte count at few intervals could be due to the variations of plasma heavy metal levels and variations in O₂ supplies as well as due to the variations in depositions of heavy metals in the skin, kidney, liver. Menkin (1955) isolated two polypeptides, thermolabile and thermostable in the protein fractions of cell exudants and showed that these were the leucocytosis producing factors. In the present research the cellular exudents are released as a result of necrosis and these exudents may contain the leucopoietic factors to
promote leucopoiesis. The sudden rise in these factors along with other leucopoietic factors could lead to a sudden spurt of WBC count at a few intervals. But it needs further investigation to find out how mining effluents induce elevation of leucopoiesis in tadpoles.

**ALTERATIONS OF WBC DIFFERENTIAL COUNT:**

The mining effluents induce significant changes of the lymphocyte and neutrophil counts of the tadpoles of various stages.

**LYMPHOCYTE:**

Mining effluents (ME) induce concentration dependent and to a certain extent exposure period dependent rise of lymphocyte counts of tadpoles of stage V. Especially at the end of one twenty hours all the concentrations of ME induce sharp rise of lymphocyte counts. ME induces concentration dependent and moreover exposure period dependent elevations of lymphocyte counts of tadpoles of stages VI to VIII but all the concentrations of ME except 10.0 % promote a slight decrease of lymphocyte count at the end of twenty four hours. All the concentrations of ME induce concentration dependent elevations of lymphocyte counts but 0.01, 0.1 and 5.0 % ME induce fluctuations of lymphocyte numbers, of tadpoles of stages from XIX to XIV. But Me induces sharp elevations of lymphocyte count. 0.01 % ME induces progressive increase of lymphocyte count up to the end of forty eight hours but subsequently drops the lymphocyte count sharply up to the end of one twenty hours. Similarly 0.1 % ME reduces lymphocyte counts sharply at the end of one twenty hours. ME induces concentration dependent elevations of lymphocyte counts of tadpoles of stages
from XIX to XXII at the end of six hours but subsequently all the concentrations of ME except 10.0 and 30.0 % promote fluctuations of lymphocyte counts.

**NEUTROPHIL COUNTS:**

ME induces concentration and exposure dependent decrease of the neutrophil counts of the tadpoles from stages I to XIV, especially the neutrophil counts drop sharply up to the end of one twenty hours. But in tadpole stages from IX to XIV ME induces sharp decline of neutrophil counts at the end of forty eight hours to elevate steadily up to the end of ninety six hours. However though neutrophil counts are elevated they are far below to those observed in control tadpoles. Similarly 0.01 % ME induces drop in neutrophil count upto forty eight hours in tadpoles of stage VIII, and then the neutrophil counts increase steadily up to the end of one twenty hours, however the neutrophil count remains quite below the normal level. Rest of the ME concentrations induce steady decrease of neutrophil counts in an exposure and concentration dependent manner. Interestingly 0.01 % ME induces slight increase of neutrophil counts of tadpoles of stages XIX to XXII at the end of twenty four hours only. ME concentrations like 0.01 to 5.0 % promote fluctuations but reductions of neutrophil counts. ME concentrations like 10.0 and 30.0 % induce sharp reduction of neutrophil counts at the end of six hours and then steadily decline further.

Interestingly all the stages of tadpoles show the presence of lymphocytes and neutrophils only. There is hardly any report on the heavy metal induced alterations of the tadpoles' differential count but the heavy metal induced alterations of the WBC differential counts have been reported for fishes. Shandilya and Banerjee (1989) reported increase of lymphocytes of fishes exposed
to zinc and chromium. Rao et al., (1990) have observed increase of lymphocyte count and leucocytosis under the synergistic action of Pb, Cd, Cu, Fe, Zn and Mn in fishes inhabiting polluted waters. Therefore, rise of lymphocyte count could be considered as the effect of heavy metals on the leucopoietic tissue as mining effluents bear heavy metals but it is difficult to judge which of the heavy metal/s are promoting lymphocytosis. The drop in neutrophil counts be considered as a negative reaction to lymphocyte count or a compensatory action. It may also be due to the decrease of neutropoietin, a factor responsible for neutrophil formation, under the influence of mining effluents. A sudden spurt in neutrophil counts could be due to the sudden release of neutropoietic factors during necrosis (Chatterjee, 1977). The fluctuations of lymphocytes/neutrophil numbers could be due to the fluctuations in blood retentions of heavy metals and degree of necrosis as well as release of leucopoietic factors.

However, it needs further investigation to throw more light on the actions of mining effluents on the leucopoietic tissue of tadpoles and differentiation of leucocytes.

**Alterations of Blood Platelets:**

ME induces increase of blood platelet counts independent of ME concentration and exposure periods in tadpoles from stage I to V. But 0.1 % ME induces decrease of blood platelet count at the end of twelve hours while at the end of twenty four hours 0.01, 0.1, 1.0 and 30.0 % ME induce decrease of blood platelet counts. At the end of forty eight, seventy two and one twenty hours 0.01 % ME induces decrease of blood platelets counts while rest of the doses induce rise and fluctuations of blood platelet counts. ME induces concentration
dependent (except 0.01 %) and exposure period dependent elevations of blood platelet counts in the tadpole stages from VI to VIII. Only 0.01 % ME induces reduction of blood platelets counts at the end of one twenty hours. However all the concentrations of ME (except 0.01 %, 0.1 %) induce reductions and fluctuations in blood platelets counts of tadpoles of stages from IX to XIV but 0.1 % ME induces elevations of blood platelets counts at the end of twelve, twenty four, forty eight and ninety six hours. But ME induces significant elevations of blood platelet counts above the normal level at almost all the exposure periods in tadpole stages from XV to XVIII. Only 0.01 and 0.1 % ME induce decline of blood platelet counts at the end of one twenty hours. Interestingly all the concentrations of ME induce concentration and exposure period dependent sharp elevation of blood platelet counts of the tadpoles of stages from XIX to XXII.

It is known that the blood platelets are useful for repairs of capillary endothelium, initiation of blood clotting and assisting haemostatic mechanism. The ME induces necrosis of the kidney, skin, liver, intestine, glomerular capillaries and may also be causing injuries to some other tissues which result in bleeding. The increase of the production of blood platelets may be in response to the necrosis and bleeding in order to effectively clot the blood, repair the capillary endothelia of glomeruli. Therefore, the rise and fluctuations of the blood platelet number may be considered due to the variations in the degree of necrosis and bleeding as well as partly due to the variations in the activation of megakaryocytes like tissues of tadpoles responsible for the formation of blood platelets. The decrease of the blood platelets could be due to the increased absorption and deposition of heavy metals from the mining effluents in the tissue.
responsible for the formations of megakaryocytes like cells. Deposition and toxic action of heavy metals may inhibit the process of blood platelet formation which result in the decrease of blood platelet numbers. The fluctuations of blood platelet counts could be due to the fluctuations in the depositions of heavy metals, their elimination from the blood and the degree of inhibition of haemopoietic tissues related to the megakaryocyte formations, especially the stem cells like cells. There is hardly any report on the heavy metal induced alteration of the blood platelet counts of any animal. However, Bandiwdekar (1996) studied the effect of mining effluents on the blood platelet counts of mallard-ducks where he observed rise in fluctuations in blood platelet counts under the influence of various concentrations of ME and these changes were independent of ME concentrations but were dependent on necrosis of kidney and other tissues. The kidney is known to haemopoietic tissue of tadpoles (Wassersug, 1997) and the degree of necrosis of Kidney would affect the platelet count as in the present work, ME is found to induce necrosis of kidney.

**BIOASSAY OF PLASMA:**

**ALTERATIONS OF TOTAL PROTEIN CONCENTRATIONS:**

All the stages of tadpoles get equally influenced by the mining effluents. Mining effluents (ME) induce concentration and exposure period dependent elevations of the total protein concentrations of the plasma. Low concentrations of ME induce comparatively less elevations of plasma protein concentrations if compared with the elevations promoted by higher concentrations of ME but if compared with the total protein concentrations of the plasma of control tadpoles the elevations of the plasma protein levels induced by 0.01 % ME are highly
significant and as exposure period increases there is successive increase of plasma total protein levels.

In the present investigation mining effluents (ME) are found to induce necrosis of skin, intestine, liver and kidney and may also be inducing injury to the other organs. The rise of plasma total protein concentrations may be considered as due to the necrosis of skin, intestine, liver and kidney as well as due to the injury to other organs and the proteins from the necrotic tissues may be passed down the blood as per Merill's hypothesis (1956), thereby increasing the plasma protein levels. All the concentrations of ME induce necrosis and the degree of necrosis depended upon the exposure period, therefore the plasma protein levels elevate for all concentrations of ME and increase progressively as the exposure period increases. Slight variations observed in the plasma protein levels at a few exposure periods and tadpole stages could be due to the related variations in the degree of necrosis, number of cells under going necrosis and passing of some proteins against the concentration gradient into the extra cellular spaces. Unfortunately there is hardly any report on the heavy metal induced or mining effluents induced alterations of the plasma proteins of the tadpoles and frogs in general. Kaplan et al., (1967) recorded reduction in erythrocyte count of R. pipiens under the influence of lead and in the present investigation the decrease in the red blood cell count is observed under the influence of ME as the exposure period increases. Thus the destruction/degeneration of RBCs could also contribute to the exposure period dependent elevations of plasma proteins. Bandiwdekar (1996) has observed mining effluents induced elevations of total proteins of the plasma of ducks and the elevations were reported to be concentration dependent.
ALTERATIONS OF ACID PHOSPHATASE:

The control tadpoles exhibit successive increase of AP activity from the end of six hours to the end of one twenty hours but mining effluents induce concentration and exposure period dependent significant increase of the AP activity of the plasma of all the stages of tadpoles. All the stages of tadpoles except VI to VIII exhibit a distinct ME concentration effect on the AP activity of the plasma but the plasma of the tadpoles stages VI to VIII exhibit a narrow difference of the AP activity under the influence of various concentrations of ME though ME concentration effect is evident. The tadpoles of stages XV to XVIII exhibit a very pronounced elevation of AP activity under the influence of 30.0 percent ME.

The rises of the plasma AP activity may be occurring due to the progressive necrosis during which AP passes down to the blood, thereby increasing plasma AP levels (Merin et al., 1956). In the present investigation ME induced necrosis of skin, intestine, liver and kidney of all the tadpole stages has been observed and these necrosis are ME concentration and exposure period dependent. Role of AP in necrosis of tissues is well documented. During necrosis AP gets released from the injured tissues. Many researchers have shown that the increase of plasma/serum AP are due to the damage to the cells of an organ. Therefore, the ME concentration and exposure period dependent elevations of the plasma AP of the various stages of tadpoles could be due to the release of AP from the necrotic tissue and its subsequent passing down into the blood. Rees et al., (1961); Fox et al., (1962); Rees and Schotlander, (1963); Slater and Greenbaum, (1965) have shown that the carbon tetrachloride induced liver necrosis promoted
rapid elevations in the serum enzyme activities due to the leakage from damaged tissues. Dingle and Fell (1968) suggested the scavenging action of AP during necrosis. The liver ischemia releases lysosomal enzymes into the plasma/serum (Duved and Beaufay, 1959; Bassi and Barnelli Zazzera, 1964; Kerr, 1965). Baccino et al., (1965), Alpers and Isselbacher (1967) have reported the release of lysosomal enzymes during liver injury. Clinical studies have shown correlation between the severity of the diseases and the degree of enzyme activities in the serum. Elevations of AP levels in the blood circulation after the tissue injury have been reported by Janoff et al., (1962). Badiwdekar (1996) observed increase of serum AP activity of the ducks under the influence of mining effluents and attributed it to the degree of necrosis induced by ME to the liver and kidney. Therefore, on the basis of above reports, it can be inferred that the progressive elevations of plasma AP activity could be due to the leakage of AP activity from the necrotic tissues of the tadpoles under the influence of mining effluents. It is worth to note that several researchers have identified the AP activities in the blood and other tissues of amphibians and frog tadpoles in particular (Ponz, 1947; Krugelis, 1949-1951; Cafiero et al., 1950; Kind and Macchi, 1952; Burgos, 1955; de Cesaris-Coromaldi, 1955; Longley, 1955). However, none of them has studied AP activity of tadpoles under the influence of either heavy metals or mining effluents.

**ALTERATIONS OF NON-SPECIFIC-ESTERASE:**

The control tadpoles showed a very low activity of NSE of plasma and NSE activity slightly elevates as the tadpoles grow. Mining effluents (ME) induce concentration and exposure period dependent significant increase of the NSE activity of the plasma of all the stages of tadpoles. However, ME fails to induce a
vast difference in the NSE activity under the influence of successive concentrations of ME though ME concentrations effect is promoted.

The ME concentration and exposure period dependent progressive elevations of the plasma NSE activity of all the stages of the tadpoles could be explained on the basis of acuity of necrosis as well as progress of necrosis along with the involvement of number of cells/tissues in necrosis. Like AP, NSE is also a lysosomal enzyme. During necrosis, the NSE being lysosomal be getting released or leaked from the necrotic skin, intestine, liver and kidney of the tadpoles, thereby passing down into the blood circulation (Merill et al., 1956) causing elevation of plasma NSE activity. Other researchers have also suggested the release of lysosomal enzymes from the necrotic tissues to the blood (Zelman et al., 1959; Janoff et al., 1962; Alpers and Isselbacher, 1967). Bandiwdekar (1996) observed progressive elevations of NSE activity of the serum of ducks exposed to mining effluents.

Interestingly, the pattern of changes of the NSE activity of plasma of tadpoles exposed to ME is almost identical to that observed for the AP. As both the AP and NSE are lysosomal enzyme and lysosomal enzyme activities are known to elevate during necrosis (Dingle and Fell, 1968-1973), it can be inferred that elevations of NSE activity and its progression observed in the plasma of tadpoles are due to the necrosis of different tissues of tadpoles under the influence of heavy metals from the mining effluents as these enzyme gets released from the necrotic tissue into the blood.

**ALTERATIONS OF ALKALINE PHOSPHATASE ACTIVITY (AIP)**:
The mining effluents induce concentration and exposure period dependent elevations in the AIP activity of the plasma of tadpoles of all the stages i.e. from I to XXII. Interestingly the pattern of alterations of AIP activity of plasma of various tadpole stages exposed to mining effluents was almost identical. However, ME could not induce pronounced elevations of the AIP activity of the plasma at the end of six hours in all the tadpoles of different stages but at the end of one twenty hours, AIP activity of the plasma elevated considerably in all the tadpoles. At every exposure period, though ME induced concentration dependent elevations of the plasma AIP activity, the differences in AIP activity under the regimen of different ME concentrations were very narrow.

The present investigation has shown the induction of ME concentration and exposure period dependent necrosis of skin, intestine, liver and kidney as well as reduction of the erythrocyte number which partly could be due to the destruction/degeneration of some erythrocytes. The rise in the AIP activity of plasma is well documented (Keele et al., 1992). Ponz (1947) reported AIP activity from the liver, pancreas, lung, intestine and bile of embryonic to larval stages of Bufo vulgaris. Krugelis (1949-1951) showed AIP activity in all the tissues of A. mexicanum and X. laevis. Cafiero et al., (1950) demonstrated AIP activity in different tissues of Triton cristatus. Kind and Macchi (1952) studied the AIP activity from kidney, heart, liver, spleen and muscle of R. pipiens while de Cesaris-Coromaldi (1955) studied AIP activity from the embryonic tissues and from tadpoles of Bufo vulgaris and showed that AIP activity is more in caudal than in cephalic regions. Similarly Burgos (1955) showed the AIP activity in Rana papiens, histochemically. Therefore the elevations of the AIP activity of the plasma
of all the tadpole stages of *R. tigerina* could be considered due to the necrosis of skin, intestine, liver and kidney as well as other organs under the influence of ME and several researchers have clearly demonstrated the presence of AlP activity in several tissues of many frog species. The progressive increase of AlP activity of plasma could be due to the progressive necrosis induced in different organs of tadpoles by ME in accordance with different concentrations and exposure periods. Bandiwdekar (1996) has demonstrated elevations of the AlP activity of the serum of ducks exposed to mining effluents. Hence successive rises of plasma AlP activity could be due to the release of AlP from the necrotic tissues of tadpoles into the blood circulation as suggested by Merin *et al.*, (1956).

**ALTERATIONS IN ELECTROLYTES:**

**SODIUM:**

Mining effluents induce concentration and exposure period dependent elevations of the plasma sodium concentrations of the tadpoles of stages I to V. Especially elevations of the sodium levels reach peak levels at the end of ninety six hours to decline sharply at the end of one twenty hours. Almost similar pattern of changes of the plasma sodium levels are promoted by ME in rest of the tadpole stages but in the stages VI to VIII 0.01 to 1.0 percent ME induce decrease of plasma sodium levels at the end of six hours while at the end of one twenty hours sodium plasma level drops under the influence of 0.01 and 0.1 % ME. ME induce concentration dependent increase of the plasma sodium levels of the tadpoles of stages from IX to XVII at the end of six hours but subsequently it reduces slightly or elevates slightly at the end of twelve hours and then generally elevates successively up to the end of ninety six hours to reduce once again.
sharply. Low concentrations of ME induce comparatively sharp decline than the larger concentrations of ME. However plasma Na⁺ levels are seen above the control levels. ME promote concentration dependent elevations of the sodium concentrations of plasma of tadpoles of stages from XIV to XXII at the end of six hours. Then the pattern of changes of the sodium concentrations of plasma of tadpoles remain nearly same. Kanno et al. (1978) have reported altered permeability of epithelial cells of newt stomach under the influence of zinc. Arhem (1980) observed altered permeability of sodium ions in myelinated nerve fibres of Xenopus laevis. Fromm (1981) examined osmoregulation across Rana pipiens skin in vitro at various pH levels and observed decreased flux of sodium ions into the skin, reducing overall transport. However, in the present investigation the necrosis of skin, intestine, liver and kidney has been observed and as a result the sodium ions from the necrotic tissues would be released into the blood as per Merill’s (1956) hypothesis. The number of ions leaking or releasing from the necrotic tissues into the blood would depend upon the degree of necrosis and not upon the altered permeability of epithelial cells or integument/skin of tadpoles. Therefore, the ME concentration dependent elevations of the plasma sodium levels could be considered due to the release of sodium ions into the blood depending upon the degree of necrosis of tissues which has been observed to be dependent upon the concentrations of ME and exposure periods. The decrease of the sodium concentration of the plasma could be attributed to the leakage of some sodium ions into the extra cellular spaces as well as their elimination by the kidney of tadpoles due to the impairment of renal
functions and renal regulations of ions by the skin which acts as an endocrine organ in tadpoles (Wassersug, 1997).

**POTASSIUM:**

The potassium levels of the plasma of control tadpoles of stages from I to XXII show steady increase while potassium levels in the plasma of control tadpoles of stages XXII to XXV shoot up from the end of forty eight hours to the end of one twenty hours.

Interestingly mining effluents induce concentration and exposure dependent elevations in the potassium levels of plasma of all the stages of tadpoles and only at the end of one twenty hours ME induce decline of potassium levels of plasma. Tadpoles of only stages XXII to XXV promote decline of potassium levels of plasma below the control level at the end of one twenty hours under the influence of 0.01 % ME. Rest of the concentrations of ME though decline potassium levels of plasma at the end of one twenty hours, they fail to bring the potassium level below the control levels and the decline of potassium levels is such that relatively higher doses decline K⁺ concentrations more than the higher concentrations of ME indicating the decline in effectiveness of lower concentrations of ME at the end of one twenty hours. In case of tadpoles of stages I to V, 0.01 % ME can induce elevations of potassium level of plasma at the end of six hours to decline it slightly at the end of twelve hours and subsequently it shoots up K⁺ concentration steadily up to the end of forty eight hours to remain unchanged up to the end of one twenty hours.

The present investigation shows that ME induces almost identical pattern of potassium concentration changes of the plasma of all tadpole stages of *Rana*.
tigerina. Arhem (1980) has reported zinc induced decrease in the kinetics of potassium system of alterations of K+ levels in myelinated fibres of Xenopus laevis while Kanno et al. (1978) reported alterations of potassium kinetics of epithelial cell membranes of the newt stomach and he attributed it to zinc combining with sulfhydryl groups. As mining effluents contain several heavy metals, they are likely to alter the potassium permeability of the epithelial cells of several organs/tissues by combining with sulfhydryl groups of the membranes. It may lead to alterations in potassium permeability. Based on the changes of potassium permeability the plasma potassium levels may elevate or decline. Besides this mining effluents (ME) are inducing progressive necrosis of the skin, intestine, liver and kidney which may result in progressive release of potassium from the necrotic tissues depending upon the degree of necrosis induced. It is also observed in the present investigation that ME induces necrosis of these organs in a concentration and exposure period dependent manner. The elevations of the potassium levels of plasma are also showing dependency on the ME concentrations and exposure periods. Therefore, elevations of the potassium levels of plasma of tadpoles could be considered due to the necrosis of tissues which releases/leaks potassium ions that get down to the blood circulation as per Merill's (1956) hypothesis. The contribution of disturbed membrane permeability in elevating potassium levels of plasma cannot be ruled out. The decreases in potassium concentrations of plasma could partly be due to the elimination of potassium ions by the injured kidney, its failure to reabsorb potassium ions, insufficient operations of ion regulatory mechanism and partly due to lesser leakages of necrotic tissues as the time passes as well as due to the release of some
potassium ions in the extra cellular spaces. Thus, it can be concluded that mining effluents induced necrosis plays a major role in the elevations of plasma potassium concentrations of the tadpoles. Bandiwdekar (1996) observed ME induced elevations of serum potassium levels of ducks.

**ALTERATIONS OF PLASMA CALCIUM CONCENTRATIONS:**

The present investigation shows that all the control tadpoles of all the stages except VI to VIII promote steady and marginal elevations of the plasma calcium concentrations. Only in case of tadpoles of stages VI to VIII promote sharp progressive increases of the plasma calcium levels from the end of twelve hours to the end of one twenty hours. It is also clear from the present investigation that mining effluents induce ME concentration and exposure period dependent manner elevations in calcium levels of plasma of all the tadpole stages. Though, calcium concentrations of the plasma of tadpoles elevate in ME concentration and exposure period dependent manner, the differences in the elevated calcium levels induced by different concentrations of ME are narrow with respect to each other. 0.01 ME fails to induce increase of plasma calcium level beyond the level found in control tadpoles at the end of seventy two to one twenty hours. In general 30.0 % ME induces highest elevations in the calcium concentrations of plasma of all the tadpole stages.

Unfortunately, there is hardly any report on the heavy metal induced or mining effluent induced changes of the calcium levels of tadpoles or frogs. However Bandiwdekar (1996) observed mining effluent induced elevations of the serum calcium levels of the ducks and he attributed these elevations to the leakage/release of calcium by the necrotic kidneys into the blood.
Therefore, the elevations of calcium levels of plasma of different tadpole stages under the influence of mining effluents could be attributed to the necrosis of skin, intestine, liver and kidney. In the present investigation the necrosis of skin, intestine, liver and kidney has been observed to depend upon the exposure period and mining concentrations. As a result of such necrosis cytoplasmic calcium ions, calcium bound to the proteins, calcium of mitochondrial and endoplasmic reticular origin are likely to be swept/released into the tadpole blood circulation (Merill et al., 1956) thereby elevating the plasma calcium level.

Calcium is stored by Amphibians in unique structures, the paravertebral lime sacs (Schlumberger and Burk, 1953). These gland like structures envelope the spinal ganglia and contain calcium to the extent of over 90% calcium carbonate in the form of the mineral, aragonite. Studies with intact Bufo bufo larvae and Ca-lactate have shown that the calcareous deposits of the lime sacs are used for ossification and a decrease in the CaCO3 in these sacs occurs during growth and mineralization of the skeleton which accompanies metamorphosis (Guardabassi, 1960). Therefore, the necrosis of these lime sacs under the influence of mining effluents may release more calcium into the blood circulation which could elevate plasma calcium level depending upon the degree of necrosis.

Alterations of Plasma Chloride Levels:

The present investigation shows that the chloride levels of the plasma of control tadpoles of stages I to VIII and XV to XVIII remain almost unchanged up to the end of seventy two hours and then elevate marginally up to the end of one twenty hours, while the chloride levels of the control tadpoles of stages IX to XIV and XIX to XXV remain almost unchanged up to the end of twenty four hours but
subsequently keep on rising up to the end of one twenty hours. The present investigation clearly shows that mining effluents induce nearly concentration dependent and exposure period dependent elevations of plasma chloride levels of almost all the stages of tadpoles except stages VI to VIII where 0.01 % Me induces decrease of chloride concentrations of plasma from the end of six to twenty four hours only. It is also revealed that all the concentrations of ME induce sharp elevations of the concentrations of chloride of plasma from the end of forty eight hours to the end of one twenty hours in all the stages of tadpoles. The present investigation also reveals that all the concentrations of ME induce almost identical pattern of plasma chloride elevations in almost all the tadpole stages.

There is hardly any report on the heavy metal induced or mining effluent induced changes of the chloride concentrations of tadpoles. However, Bandiwdekar (1996) observed elevations in the chloride concentrations of the plasma of ducks exposed to mining effluents and he attributed these elevations to the leakage of chlorides from the necrotic tissues in to the blood circulation. Chlorides have been known as chief anions of extracellular fluid, esophageal secretions, gastric HCl and several tissues and are known to be associated with water, acid-base balance and neuromuscular activity (Brown, 1964).

The elevations in the chloride concentrations of the plasma of different tadpole stages under the influence of mining effluents could be due to the necrosis of skin, intestine, liver and kidney. The present investigation shows that mining effluents induce concentration and exposure period dependent progressive necrosis of skin, muscle associated with skin, liver, intestine and
kidney and the chlorides present in these tissues could be released/leaked in the blood circulation thereby elevating plasma concentrations of Cl⁻ ions. As the necrosis progresses there could be progressive release in the Cl⁻ ions from the tissue to the blood. Besides this the failure of the injured kidney/necrotic tubules to absorb enough Cl⁻ ions could result in elevating the Cl⁻ ion concentrations from the plasma. Also, the necrotic tubules may promote more elimination of Cl⁻ ions through urine as well as some Cl⁻ ions may be passed in to extracellular spaces resulting in the decrease in chloride ions at some time intervals/exposure periods. Thus, it is concluded that the mining effluents can induce elevations in the chloride concentrations of the plasma of different stages of tadpoles.

**BIOCHEMICAL ANALYSIS:**

**PROTEINS:**

The present investigation shows that the protein levels in the skin, intestine, liver, kidney and muscles of the control tadpoles of all the stages exhibit a steady and progressive increase from the end of six hours to the end of one twenty hours. It is obvious that the protein levels in these organs would steadily increase as the tadpoles are developing rapidly in volume, size and weight which is possible due to the increased protein synthesis and addition of new proteins to carry out normal metabolic activities. The present investigation reveals that mining effluents induce reduction of the total protein levels of the skin, intestine, liver, kidney and muscles of all the stages of tadpoles in accordance with the concentrations of mining effluents and the length of exposure periods. The ME
can induce progressive decrements of the total protein levels of all the organs mentioned above of all the tadpoles stage as the length of exposure period increases. The mining effluents like 0.01 % can induce minimum reduction in the total protein levels of skin, intestine, liver, kidney and muscles of all the tadpole stages (I to XXV) at the end of six hours and the decline of total protein levels successively increase thereafter till the end of one twenty hours, while 30.0 % ME induces maximum reduction of the total protein levels of skin, intestine, liver, kidney and muscles of all the tadpole stages at the end of one twenty hours. These changes i.e. reductions of the total protein levels of skin, intestine, liver, kidney and muscles of all the tadpole stages could be attributed to the progressive necrosis of these tissues. In the present investigation the necrosis of the skin, muscles associated with the skin, liver, intestine and kidneys are observed and the degree of necrosis is observed to be dependent upon the concentration of mining effluents and the length of exposure periods. As the above referred tissues are getting mild to acutely necrotic, the cells of the effective tissues lose their integrity and some are sloughed off. Their cytoplasmic constituents gets broken down by the action of lysosomal enzymes as evidenced by the biochemical assays. Besides this the cytoplasmic debris is exuded in the lumina or interstitial spaces or interstitium (kidney) and the proteins pass down the tissue into blood circulation. The progressive increase of plasma protein levels substantiates this. Thus, the necrosis of skin, intestine, liver, kidney and muscles of different tadpole stages, probable loss of proteins through urine and faeces, its passing down the concentration gradient into the extracellular spaces as well as blood may be responsible for the decline of total protein levels. Similar findings
have been reported by Bandiwdekar (1996) while working on actions of mining effluents on the ducks.

**GLYCOGEN:**

The present investigation shows that the glycogen contents of the skin of the control tadpoles of different stages increase successively and marginally from the end of six hours to the end of one twenty hours. The elevation of glycogen concentration of skin of tadpoles from the end of six hours to the end of forty eight hours is quite insignificant. The present research reveals that mining effluents induce concentration and exposure period dependent manner, progressive increase of the glycogen concentrations of skin of all the tadpoles stages under investigation. Therefore ME concentration like 0.01 % can induce minimum elevation of the glycogen concentration of the skin of all the stages of tadpoles at the end of six hours and it continues to elevate glycogen concentration up to the end of one twenty hours, while 30.0 % ME induces maximum increase of the glycogen concentrations of the skin of all the stages of tadpoles at the end of one twenty hours. It is known that anuran skin contains a vast pharmacopoeia of neuroactive and vasoactive peptides (Erspamer, 1994). As anuran skin is an endocrine organ it could release its hormonal products into the circulatory system in direct response to external influences on the skin. Also it is known that sauvagine (Corticotropin- releasing hormone) in the central nervous system of tadpoles plays a key role in regulating metamorphosis (Denver, 1996-1997). But sauvagine is also found in high concentration in the skin of some anurans (Erspamer, 1994). Wassersug (1997) suggests that the possibility of the release of sauvagine from the injured skin of the anurans need to be tested. It is well
documented that tadpole skins are sensitive to the chemicals in their surroundings (Relya, 1995; Wassersug 1997). Recent work has shown that tadpoles in response to chemicals or stress signals suppress the feeding. Therefore, it is possible that under mining effluent stress, the endocrine activities of the skin of tadpoles gets activated to produce several hormonal products to cope up with the disturbances in ion regulation. Skin permeability, electrical potential, ion kinetics and changes of the structural units of membrane protein or skin protein, reported in amphibians by several authors under metal toxicity or pH stress (Kanno et al., 1978; Celentano et al., 1979; Fromm, 1981). This stepping up of endocrine activities of skin would require elevation of metabolic rate and to meet the energy demand or elevation of metabolic rate ample elevation of nutrient sources is essential. Therefore, such high demand of nutrient source (i.e. glucose) could elevate mobilisation of glucose/glycogen from different organs which may result in accumulation/elevation of glycogen contents of the skin which could result in the depletion of glycogen levels from the natural glycogen stores like liver and muscle. In the present investigation the depletion of liver and muscle glycogen of all the tadpole stages is observed which substantiates this possibility. Misyura (1996) also has reported elevation of glycogen levels of skin of amphibians exposed to heavy metal pollution. Therefore, the progressive elevations of glycogen concentrations of skin of tadpoles may be considered as a stress response to mining effluents as mining effluents induce progressive necrosis of skin and other organs.

The glycogen concentrations of the liver and muscles of the control tadpoles of all the stages show steady increase from the end of six hours to the
end of one twenty hours. This increase of glycogen levels in control tadpoles could be considered as a measure of step up glycogenesis in liver or muscle as well as mobilisation of carbohydrates in order to increase its glycogen stores/stocks for the future growth and metamorphosis as both these events require huge energy budget.

The present investigation reveals that mining effluents reduce the glycogen levels of both the liver and muscles of all the tadpole stages in a ME concentration and length of exposure period dependent manner. Therefore, the depletion of glycogen concentrations in these tissues could be due to the necrosis of these tissues induced by mining effluents and partly due to the mobilisation of glycogen from these organs to the skin which acts as an important endocrine organ demanding more cellular fuels like glucose/glycosyl units to cope up with mining effluent induced stress. Misyura (1996) has reported depletion of liver glycogen contents of amphibians under the stress of heavy metals. Thus it can be concluded that the ME concentration dependent and exposure period dependent reductions in the glycogen levels of the liver and the muscle could be due to the progressive necrosis of these organs and the mobilisation of glycogen from these organs to skin. The elevation of skin glycogen contents substantiate this.

**ALTERATION OF AP ACTIVITY:**

The present work shows that the control tadpoles of all the stages have very low AP activity in skin, liver, intestine, kidney and muscle, at all the exposure periods but as the tadpoles get exposed to varying concentrations of mining effluents (ME) for various lengths of time the AP activity from the different organs of the various stages of tadpoles shoot up. ME could induce
concentration dependent and exposure period dependent elevations of the AP activities of skin, intestine, liver, kidney and muscles of the tadpoles of stage V at the end of six and twelve hours or in few organs for a period ranging from six to one twenty hours. However, the different organs of all the tadpole stages except V could not show ME concentration dependent and exposure period dependent elevations of AP activity. In most of the tadpole stages the AP activity elevated sharply at the end of one hundred and twenty hours under the influence of 0.01 or 30.0 % ME in general and in a few cases under the influence of 10.0 or 0.1 % ME.

The elevations of the AP activity of different organs of different tadpole stages under the mining effluents could be attributed to the induction of necrosis in these organs. The apparent variations in the degree of elevations of AP activity in different organs of various tadpole stages under the influence of ME could be due to the variations in : the degree of necrosis, progress of necrosis, release of AP from these organs in to the blood circulation as well as into the extracellular spaces and the elimination of AP through urine. Generally necrosis once set in it progresses gradually and also keeps on involving more cells but in order to protect the other unaffected tissue the body tries to flush out/eliminate the AP or other proteolytic enzymes from the site of injuries. These processes would result in depletion of AP activity but as the exposure of organs to ME continues some other tissues may begin to undergo necrosis and depending upon the stage of necrosis, AP activity would appear to be elevated. Hence, concentration and exposure dependent rises in AP activity in these organs could not be observed though the AP activity increases as the necrosis progresses.

30.0 % ME elevates
AP activity very significantly and alarmingly as it can induce necrosis in relatively large number of tissues/cells as the exposure length increases. But the excessive rise of AP activity under the influence of 0.01 % ME is more puzzling. Bandiwdekar (1996) observed similar rise of AP activity under the influence of 0.01 % ME in the kidney of ducks. The association of AP activity and necrosis is well established (Slater and Greenbaum, 1963). Slater and Greenbaum,(1963) observed that increase of AP activity of the kidney is proportional to the degree of necrosis. Under the influence of ME, AP activity increases in proportion with the progress of necrosis as evidenced by its elevation of the blood plasma. Several researchers have reported the role of AP in necrosis of several tissues. Becker and Barron (1961) reported increase of AP activity in injured neurons. Gould and Holt (1961), Kawai (1963), Holtzman and Novikoff (1965) and Friede (1966) have reported increase of AP activity in the injured axons. Dianzani (1963) reported increased AP activity in the injured liver. The elevations of AP activity during necrosis and the role played by AP in necrosis are well documented and a vast literature is available on this aspect (Lysosomes in Biology and Pathology. Vol. 1 to 5 edited by Dingle and Fell Dean, Vol. I - 1969, Vol. II - 1969, Vol. III - 1973, Vol. IV - 1975 and Vol. V -1976). Sztriha et al., (1975) have opined that AP activity elevates to destroy the damaged cell/constituents. Dingle and Fell (1969) have shown the scavenging role of AP activity in diseased tissues. Kobayashi et al., (1971) reported high level of AP activity in damaged/injured tissues.

It may possibly be that during necrosis some of the lysosomes get lost or the lysosomal enzymes from the cytoplasm may get passed on to the lumen
(intestine and renal tubules) along with the cytoplasmic debris, or washed into the surrounding water bodies or internal tissue fluid from the skin as skin of tadpoles is in direct contact with water and also is known to absorb water continuously. This continuous flow of water may wash off the necrotic tissues up to a large extent thereby decreasing or fluctuating the AP activity of skin and muscles as well. Merill et al., (1956) put up a hypothesis of passing down of enzymes against the concentration gradient into the extra cellular space and blood. The elevation of AP activity of the blood plasma substantiates this possibility, therefore it may also reduce the AP activity of different organs at different time intervals depending upon this release into the extra cellular space as well as blood, renal elimination and flushing of different organs by incoming water from the skin, faecal elimination through gut etc.

Alterations of Non Specific Esterase (NSE):

The present investigation reveals that the control tadpoles of different stages bear a marginal level of NSE activity at all the times. This work also shows that mining effluents induce concentration dependent elevations in the NSE activity of the skin of tadpoles of : stage V at the end of six hours, stage VI to VIII at all the exposure periods, stage IX to XIV at the end of six hours, stage XV to XVIII at the end of six hours, stage XIX to XXV at all the exposure periods.

During rest of the exposure periods of all the tadpole stages ME does not show concentration and exposure period effects on the NSE of skin. But at the end of one twenty hours the maximum NSE activity is induced by 0.01, 5.0, 10.0 % ME. Mining effluents' induce concentration dependent and exposure period dependent elevations in the NSE activity of the intestine of the tadpoles of : stage
VI to VIII at the end of six and twelve hours, stage IX to XIV from the end of six to forty eight hours, stage XIX to XXII at the end of six and twelve hours.

During rest of the exposure periods of all the tadpole stages, mining effluents do not show concentration and exposure period effect on the intestinal NSE activity and ME induces maximum elevations of intestinal NSE activity under the influence of 0.01, 0.1, 1.0, 5.0, 10.0 and 30.0 % concentrations at the end of one twenty hours. But mostly, 30.0 % ME induces maximum elevations in the intestinal NSE activity.

Similarly, mining effluents induce concentration and exposure period dependent elevations of the NSE activity of the liver of the following tadpole stages: stage VI to VIII at the end of six, twelve and one twenty hours, stage XV to XVIII at the end of six hours. Mining effluents do not show ME concentration and exposure period effects on the NSE activity of the liver but induce maximum elevations of the NSE activity of the liver under the influence of 0.1, 1.0, 10.0 and 30.0 % concentrations at the end of one twenty hours.

The present investigation shows that mining effluents induce concentration and exposure period dependent elevations of the NSE activity of the kidney of the following stages of tadpoles: stage IX to XIV at the end of twelve hours, stage XV to XVIII at the end of six hours and stage XIX to XXV at the end of six, twelve and twenty four hours. The remaining stages of tadpoles do not show ME concentration and exposure period effects but ME induces maximum elevations of NSE activity of the kidney under the influence of 0.01, 0.1, 1.0, 10.0 and 30.0 % concentrations at the end of one twenty hours.
The study of muscle NSE activity of the different tadpoles stages indicate that mining effluents induce concentration and exposure period dependent elevations. The following stages of tadpoles show ME concentration and exposure period dependent elevations of the NSE activity of the muscle: stage I to V at the end of six and one twenty hours, stage VI to VIII at the end of six to twelve hours, stage IX to XIV at the end of six, twelve and twenty four hours, stage XV to XVIII at the end of six, twelve, forty eight, seventy two hours and stage XIX to XXV at the end of six, twelve, seventy two, ninety six and one twenty hours. The remaining stages of tadpoles do not show the concentration and exposure period effects of ME on the NSE activity of muscle. However, ME induces maximum elevations of NSE activity of muscle under the influence of 5.0, 10.0 and 30.0 % concentrations at the end of one twenty hours.

The concentration dependent increments in the NSE activity could be considered as a result of progressive necrosis of skin, intestine, liver, kidney and muscles. The histological observations of necrosis support this. The association of lysosomes with necrosis and elevation of lysosomal enzyme actions are well documented (Becker and Barrom, 1961; Gould and Holt, 1961; Kawai, 1963; Holtzman and Novikoff, 1965; Friede, 1966; Diazani, 1963 and Dingle and Fell, 1969). The NSE is a lysosomal enzyme, therefore, the elevations of the NSE activity in a progressive necrosis of tissue is well in agreement with earlier reports. The fluctuations in the NSE activity could be due to the variation in the degree of necrosis, progress of necrosis, the number of cell/area getting involved in necrosis under a particular concentration of mining effluent and also partly due to the number of enzyme molecules getting flushed by the incoming water.
from the skin, the number of enzyme molecules passing down the concentration gradient into the extracellular space and blood (Merill et al., 1956) as well as on the number of enzyme molecules getting eliminated through cytoplasmic debris of kidney and intestine as they accumulate in the lumina. All these factors could be responsible for the apparent/virtual ME concentration independent elevations of the NSE activity in the tissues at various time intervals.

**ALTERATIONS OF ALKALINE PHOSPHATASE ACTIVITY (AIP):**

The present investigation reveals that the AIP activity in the skin, intestine, liver, kidney and muscles of control tadpoles of all the stages exhibit a steady elevation from the end of six to one hundred and twenty hours which is well in agreement with Krugelis' (1951) observations on *Xenopus laevis* and *Ambystoma mexicanum*. The liver and kidney of tadpoles bear relatively higher levels of AIP (AIP) activity and generally these organs are known to have higher AIP activity (Varley, 1976).

It is clear from the present work that AIP activity of the skin of the following tadpole stages elevate in a mining effluents (ME) concentration and exposure period dependent manner: stage VI to VIII from the end of six hours to the end of one twenty hours, stage IX to XIV at the end of six hours, stage XV to XVIII at the end of six, twenty four, forty eight and seventy two hours, stage XIX to XXII at the end of twelve and twenty four hours. In rest of the stages AIP activity elevated maximally at the end of one twenty hours under the influence of 1.0, 10.0 and 30.0 % ME but mostly 30.0 % ME induced maximum elevations of AIP activity at many occasions. At rest of the time intervals AIP activity elevations are not ME concentration dependent.
Mining effluents (ME) cannot induce ME concentration and exposure period dependent elevations of AlP activity of the intestine of all the tadpole stages, however a little exposure period effect is observed in a few cases. Mostly 30.0 % ME induces maximum elevations of AlP activity of the intestine of all the stages of tadpoles at the end of one twenty hours and in a few occasions at a few intervals/exposure periods ME concentrations like 0.1, 1.0, 5.0 and 10.0 % induce hike of AlP activity of intestine of a few tadpoles stages.

ME induces concentration and exposure dependent elevations of AlP activity of liver of the following stages of tadpoles: stage V from the end of six to twenty four hours, stage IX to XIV at the end of six, twelve and forty eight hours, stage XV to XVIII at the end of ninety six hours, stage XIX to XXII at the end of forty eight, ninety six and one twenty hours. Rest of the tadpole stages promote elevations of AlP activity independent of the strength of mining effluents. However, maximum elevations of AlP activity of liver of different tadpole stages is induced by 0.01, 0.1, 5.0, 10.0 and 30.0 % ME at the end of one twenty hours.

ME induces reduction of AlP activity of kidney below the control levels in a concentration dependent manner, except for one or two concentrations in few cases in the following stages of tadpoles: stage IX to XIV at the end of six hours, stage XV to XVIII at the end of six hours and twelve hours, stage XIX to XXII at the end of six and twelve hours. Rest of the stages promote elevations of AlP activity of kidney irrespective of the strength of ME. However at the end of one twenty hours a maximum elevation of AlP activity is induced by 0.1, 1.0, 10.0 and 30.0 % ME.
The ME concentration and exposure period dependent elevations of AlP activity and in general all the elevations of AlP activity of skin, intestine, liver, kidney and muscle of all the tadpole stages could be due to the progressive necrosis or state of necrosis promoted in these tissues as AlP activity in pathological conditions elevate in effected organs (Varley, 1976; Keele et al., 1992). Besides this the variations/fluctuations and even reductions of AlP activity of skin, intestine, liver, kidney and muscle of different stages of tadpoles could be due to the flushing of organs by incoming water, passing down of the enzyme molecules against the concentration gradient in to the extracellular space and blood (Merill et al., 1956) elimination of enzyme molecules through urine and excreta. Bandiwdekar (1996) observed similar changes of the ducks exposed to mining effluents.

**CHANGES IN ELECTROLYTES:**

**SODIUM:**

**SKIN:**

The present investigation reveals that the skin of control tadpoles of all the stages promote gradual but marginal increase of sodium concentrations from the end of six hours to the end of one twenty hours. The skin of the tadpoles of all the stages promote reduction in sodium concentrations under the influence of 0.01 % ME at the end of six and twelve hours, while at the rest of the exposure periods the mining effluents induce concentration and exposure period dependent elevations of the sodium levels. The reductions of the sodium concentrations could be due to the necrosis of the skin and the altered permeability under the influence of heavy metals in mining effluents as observed by many researchers.
while studying heavy metals toxicity to amphibians (Gosner and Black, 1957; Pough, 1976; Kanno et al., 1978; Celentano et al., 1979; Arhem, 1980; Fromm, 1981). However, the progressive necrosis and altered permeability if considered, there should be ME concentration and exposure period dependent decrease of skin sodium concentration but in the present work the sodium level appears to elevate from the end of twenty four hours to the end of one twenty hours. This could probably be due to the compensatory stepping up of homeostatic ion regulatory mechanism as sodium loss would disturb the several metabolic activities and cause an osmotic stress resulting in the death of tissue. Therefore, the skin must be stepping up the sodium transport/influx through it and also be trying to retain more sodium in the intact, non necrotic portions of the skin.

INTESTINE:

Mining effluents induce concentration and exposure period dependent increase of the sodium concentration of the intestine of all the tadpoles of all stages at the end of six to ninety six hours while at the end of one twenty hours 0.1 and 1.0 % ME induce decrease of sodium levels of intestine. The elevation of intestinal sodium level of all the tadpole stages in spite of progressive necrosis could be viewed as a compensatory sodium influx or active transport to counteract loss of sodium from the necrotic tissues to avoid osmotic crisis and ionic imbalance. Also it should be noticed that sodium is an important ion involved in several metabolic processes and physiological process like nerve impulse transmission leading to heart ion homeostasis and also active transport of solutes by the intestine. Therefore by promoting influx of sodium/active
transport or by opening sodium channel of the membranes, the tadpoles could be trying to avoid ionic crisis.

**LIVER:**

0.01 and 0.1 % ME induce reduction of sodium concentration of the liver of all the tadpoles stages at the end of six and twelve hours while rest of the ME concentrations induce elevation of Na⁺ concentration of liver. From the end of twenty four to one twenty hours all the concentrations of ME induce concentration and exposure period dependent elevations of sodium concentration of the liver of all tadpole stages. As ME induces concentration and exposure period dependent necrosis, one should expect concomitant/proportionate loss of sodium ions. However, though there could be continuous loss of sodium ions hepatic cells of the liver must be stepping up the ion transport by activating active transport of sodium ions, opening of sodium channels, to counteract the loss of ions to avoid osmotic stress, water distribution between cells, plasma and intestinal fluid (Varley, 1976). The reduction of sodium ion concentration in the early phase could be due to the altered permeability and necrotic loss of ions under the influence of ME till ion regulatory mechanism starts operating.

**KIDNEY:**

At the end of six, twelve, twenty four hours 0.01 and 0.1 % ME induce reduction of the sodium ions while 5.0, 10.0 and 30.0 % ME promote elevations of sodium concentration of kidney. However, from the end of forty eight hours to the end of one twenty hours all the concentrations of ME induce concentration and exposure period dependent elevations of sodium levels of kidney of all the stages of tadpoles. The loss/reduction of sodium concentration of the kidney
could be attributed to the necrotic loss of ions into the blood and urine and extracellular spaces but the elevations of sodium levels could be attributed to the elevated Na+ ion transport of the renal tubules and its accumulation in the intestitium to achieve maximum absorption and retention of sodium ions to avoid osmotic stress/crisis, to promote retention of water in the interstitium for the normal functioning of organ in order to counteract and negate the continuous loss of sodium ions due to progression of necrosis. Varley (1976) has suggested the role of Na+ ions in retention of water in between cells and interstitial fluid.

MUSCLES:

All the concentrations of ME induce concentration and exposure period dependent elevations of the sodium ion concentrations of the dorsal muscles of all the stages of tadpoles. Though the progressive necrosis should result in the loss of sodium ions from the muscles, the increase of sodium ion concentration of the muscles of tadpoles may be attributed to the compensatory ion regulatory mechanism. By opening the Na+ channels, and actively transporting the Na+ ions the membranes of the muscles cells could be trying to avoid the osmotic stress/crisis, impairment of the membrane electrical potential which is essential for muscle contraction and stress on metabolic activities. This also could be to retain more water in the tissue fluids and cells as sodium ions are known to conserve/retain water in the tissue (Varley, 1976).

ALTERATIONS OF POTASSIUM:

SKIN:

ME induce concentration and exposure period dependent decrease of the skin potassium level of all the tadpole stages from the end of six hours to the end
of twenty four hours and this reduction could be attributed to the loss of K+ ions by the necrotic tissue and altered permeability of the skin. Under the influence of heavy metals from the mining effluents (Kanno et al., 1978; Celentano et al., 1979; Arhem, 1980; Fromm, 1981). ME induces concentration and exposure period dependent elevations of K+ concentrations of skin of all the tadpole stages from the end of forty eight hours to one twenty hours. These elevations of potassium levels of skin could be considered as a compensatory reaction to the loss of potassium ions in the early exposure periods. These elevations of potassium levels of skin could be considered as a compensatory reaction to the loss of potassium ions on the early exposure periods. This elevations could be due to the stepping up of K+ transport by elevating active transport, opening of K+ channels and retention of K+ by the intact regions of the skin as K+ is required along with Na+ ions for the maintenance of toxic balance, osmotic pressure and normal membrane electrical potentials.

**INTESTINE:**

ME induces concentration and exposure period dependent elevations of the intestinal K+ levels of all the stages of tadpoles. Though ME concentration and exposure period dependent necrosis of intestine (intestinal villi in particular) is induced which could promote proportional loss of K+ ion, the elevations of K+ ions in the intestine could be attributed to a probable elevation of K+ transport by the intact villi by opening membrane K+ channels, increasing active transport of K+ ions in order to reduce the ionic imbalance, osmotic stress and disturbances in the membrane potentials.
LIVER:

ME induces concentration and exposure period dependent reduction of the liver $K^+$ levels of all the stages of tadpoles and this reduction of $K^+$ concentration may be attributed to the necrotic losses, elimination of $K^+$ through bile (Kaplan et al., 1967).

KIDNEY:

ME induces reduction of the kidney $K^+$ levels of all the stages of tadpoles under the influence 0.01 and 0.1 % ME at the end of six to one twenty hours. Rest of the concentrations of ME promote a little increase of the $K^+$ levels of kidney. The reduction of $K^+$ levels could be attributed to necrotic loss, impairment of $K^+$ absorption and its elimination through urine as in the present work, ME induced progressive necrosis of kidney is observed. The slight elevation of the $K^+$ level could be attributed to the compensatory elevation of $K^+$ transport/absorption by the intact renal tubules to avoid osmotic stress and impairment of membrane potential.

MUSCLES:

0.01 % ME induces reduction of $K^+$ concentrations of muscle of all the tadpole stages from the end of forty eight to the end of one twenty hours. Similarly 0.1 % ME induces decrease of $K^+$ concentration at the end of forty eight and one twenty hours. Rest of the ME concentrations induce increase of muscle $K^+$ levels. The reductions of $K^+$ levels of muscles could be attributed to the necrotic loss of $K^+$ ions and reduced permeability of the membrane of muscle.
fibres. The marginal elevation of the K\(^+\) concentration could be attributed to the improved permeability of membranes coupled with some active transport of K\(^+\) ions, opening of K\(^+\) channels to avoid osmotic stress and disturbed membrane potential. But this needs further investigation to throw more light on this aspect.

**ALTERATIONS OF THE CALCIUM LEVELS:**

Mining effluents induce concentration and exposure period dependent elevation of calcium concentrations of skin, intestine and muscle of all the stages of tadpoles at all the exposure periods, while ME induces concentration and exposure period dependent elevations of calcium levels of liver of all the stages of tadpoles from the end of six to ninety six hours and at the end of one twenty hours 0.01 % ME induces minimum elevation of calcium concentrations while 5.0 % ME induces maximum elevations of calcium. In case of kidneys of tadpoles ME induces concentration and exposure dependent elevations of calcium concentrations from the end of six hours to the end of forty eight hours. But at the end of seventy two to one hundred and twenty hours maximum elevation of Ca\(^{++}\) concentrations correlates with the degree of necrosis of all the tissues under investigation. The elevation of Ca\(^{++}\) levels could be mostly attributed to the necrosis itself as the necrosis of tissues/cells would promote release of calcium bound to the organelle and proteins and partly to the opening of calcium channels of the membranes of intact as well as injured cells under the stress of heavy metals. The elevations of Ca\(^{++}\) levels could also be due to the leakage of Ca\(^{++}\) promoted by impaired ion transport, opening of voltage gated Ca\(^{++}\) channels as observed in necrotic neurons (Pazdernik *et al.*, 1992). The ME concentrations might have elevated parathyroid and thyroid hormones from the
frog skin into the circulation which could increase cellular calcium (Howard, 1989). The amphibian (tadpole) skin is known to act as an endocrine gland bearing thyroid/parathyroid hormones along with other ion regulating hormones (Wassersug, 1997). The probable activation of calcium pump could increase the Ca\(^{++}\) levels in the cells (Guyton, 1991). Calcium is known to be pumped into the internal vesicular organelles of the cell such as into sarcoplasmic reticulum of muscle cells and into the mitochondria of all cells (Guyton, 1991). Operation of such pumps in the tissues could increase the calcium levels from the intact cells in order to compensate the loss of calcium by the necrotic cells. It is difficult to say at this juncture which of the above cited mechanism is responsible for elevation of Ca\(^{++}\) levels of the skin, liver, intestine, kidney and muscles. It is also possible that all the above referred mechanisms could be operating together to contribute to the elevation of calcium ions.

**ALTERATIONS OF CHLORIDES:**

The present investigation reveals that mining effluents induce concentration and exposure dependent manner elevations of the chloride concentrations of skin, intestine, liver, kidney and muscles of all the stages of tadpoles at all the exposure periods.

Several evidences suggests that Na\(^{+}\) and Cl\(^{-}\) are co-transported across the membranes by a carrier (Frizzell *et al.*, 1979; Ericsson and Spring, 1982). The coupled carriers are capable of producing uphill transport of one partner and the other partner moves downhill through a large gradient (i.e. secondary active transport). The coupled carrier scheme suggests that Cl\(^{-}\) can be transported into the cell. In the present investigation ME may be promoting the enhanc...
of these coupled carriers which may then elevate the Cl⁻ contents. In the present investigation ME induced concentration and exposure period dependent elevations of Na⁺ concentrations of skin, intestine, liver, kidney and muscles has been observed and these observations suggest the possibility of the operation of coupled carriers. Probably under the influence of ME this coupled carrier system is activated which may lead to more transport of chloride ions. It is established now that second messengers in the sequence of regulatory events within the cells include cAMP and Ca²⁺ ions. Cyclic AMP may activate a protein kinase that phosphorylates and opens chloride channels thereby increasing the chloride transport. It is possible that ME could activate cAMP synthesis and activating protein kinases to promote phosphorylation to open Cl⁻ channels. ME may also be influencing another chloride regulatory route of β-adrenergic which may activate phospholipase C and inositol triphosphate which increases intracellular calcium. A rise in intracellular Ca²⁺ has been shown to open Cl⁻ and K⁺ channels (Sterling, 1989).

Therefore, it can be considered that due to operations of the above mentioned mechanisms/routes the chloride levels of skin, intestine, liver, kidney and muscles of tadpoles are elevated under the influence of mining effluents. It needs further work to throw light on this aspect.

Thus it can be concluded that the mining effluents even of a very low concentration can be harmful to the developing tadpoles exposed to these effluents even for a short period. Generally the tadpoles take about 50 to 55 days for completion of all metamorphic processes to emerge out as a miniature adult frog. If these tadpoles are exposed to ME for this length of time, naturally they
would succumb to the injuries well before they metamorphose, not only this the exposure of any tadpole of early/late stage could induce deformities or severe injuries. Therefore, ME could be considered as one of the decimating factors in operation in Goa causing depletion of amphibian species. It is also important to note that the amphibian populations are declining globally.