Effects of Low Levels of Zinc on the Ovarian Development of *Tilapia nilotica* Linnaeus

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Abstract

Eight to ten days posthatch fry of Tilapia nilotica *Linn. were exposed to sublethal levels of zinc, 2 mg/l and 5 mg/l. After 30 days, ovarian differentiation occurred in the unexposed fry while gonadal anlage of zinc-exposed fry contained still undifferentiated primordial germ cells (PGC). Normal oogenesis was exhibited by the unexposed ovaries after 57 days. Zinc caused alterations in the egg membrane layers. Histopathological changes as degeneration and hyperemia in treated ovaries were observed under the light and electron microscope.*

Introduction

Rapid development of regions near freshwater areas have resulted in high levels of pollution from human, industrial, and agricultural sources. This problem calls for studies on the effects of pollutants on aquatic life, fishes in particular. Materials present in water and considered toxic have been categorized as follows: oxygen-depleting materials such as domestic wastes; toxic gases; naturally-occurring major ions of calcium, magnesium, sodium, sulfates, carbonates, and chlorides; toxic organic compounds; and heavy metals (1). Heavy metals are considered to be one of the most dangerous causing toxemias in fishes resulting to direct mortality, biological accumulation, changes in physiological function, and chronic toxicity.

Methods have been designed to investigate the effects of pollutants on fishes. In these toxicity tests, the correlation between a given concentration of the toxicant and the organism's response is investigated. Terms are used to estimate relative toxicity: median tolerance limit, TLm, where 50% of the individuals survive in the presence of a specified concentration of a toxicant usually for a specified time; lethal dose, LD_{50}, concentration of a toxicant in water which kills 50% of the individuals within specified time. An important method is to test the toxicity of sublethal levels of pollutants. Sublethal concentrations are within the normal range of adaptation in fishes but may still have adverse morphological effects interfering with health, reproduction, and survival. In the natural environment, sublethal levels of pollutants are often subtle in expression. The effects can occur unrecognized at the individual level, tolerated indefinitely, but expressed in later stages of development and interpreted at the pollution level (2). To eliminate an organism, the pollutant's concentration need not be lethal.

In terms of duration or time of exposure, toxicity tests can be classified as: acute toxicity tests, a short term exposure usually for 96 hours; chronic toxicity tests, conducted over a period of 30 days to several months; and partial chronic or adult fish partial life cycle tests. It is required that bioassays must be conducted with the most susceptible life stages of the most sensitive and dominant species. Much of the previous studies concerned lethality. The present one is on the chronic toxicity of sublethal levels of the heavy metal zinc on a freshwater teleost. Zinc is one of the metals given off by chemical industries and ore processing plants in aquatic systems (3). This investigation aims to determine the effects of sublethal levels of zinc on the histology of the developing ovary *Tilapia nilotica*. Data obtained from this study can be used in assessing quality of natural bodies of water.

Materials and Methods

Three hundred eight to ten-days posthatch fry of *Tilapia nilotica* obtained from breeders at the Natural Science Research Institute were used in the investigation.

Zinc sulphate (granular, $ZnS0_4.7H_20$, reagent grade) was dissolved in deionized water to make a stock solution for 2 ppm and 5 ppm testings. Proportions of this stock solution were introduced to diluent water to make concentrations of 2 mg/li and 5 mg/li testings. Zinc levels were monitored twice a month; zinc analysis was done with the use of atomic absorption spectrophotometer, then zinc concentrations were adjusted accordingly. Fry were acclimated first for 4 days before treatment. Sampling was done after 30 days and 57 days posthatching. One hundred fry were used per treatment and one set-up served as control.

The gonads were dissected out, fixed in 2.5% glutaraldehyde, post-fixed in osmium tetroxide, dehydrated with a series of acetone, infiltrated with propylene oxide and resin, then embedded in pure resin (Araldite), sectioned 5 micrometers thick with an ultramicrotome, stained with Toluidine blue, then observed under the light microscope.

Ultrathin sections, .001 micrometer thick were made, obtained with uranyl acetate for one hour and lead citrate for 5 minutes then observed under the electron microscope.

Observation focussed on histological features of the developing egg cells at both light and ultrastructural level. A comparative count of oogonia and oocyte stages in control and zinc-treated ovaries was done. All the developing egg cells in one transverse section taken at the same level of the ovary were counted, grouped into: oogonia, maturing oocytes, and mature oocytes. This was done for 10 ovaries.

Results and Discussion

Ovarian differentiation occurred at 30–33 days posthatching when control fry were about 12 mm. The flat cavity in the ovary can be seen (Fig. 1). Fry exposed to sublethal levels of zinc show undifferentiated gonads, consisting of only primordial germ cells and some somatic cells in the stroma. In 5 mg/li zinc-treated group, with an average total fish length of 8 mm, 1–2 primordial germ cells can be observed in the transverse section (Fig. 1), characteristic of fry at 10 days posthatching; development was delayed by approximately 20 days. In 2 mg/li zinc-treated group, with an average total length of 10 mm, 6–10 primordial germ cells can be observed (Fig. 1), characteristic of fry at 20–24 days posthatching; development was delayed by approximately 6–10 days.

In fry at 57 days posthatching, control group exhibit darkly-staining type I oocytes, large type II oocytes at protoplasmic growth and some larger oocytes at trophoplasmic growth (Fig. 2). Electron micrograph shows intact membrane layers, thecal and granulosa follicle cells with more or less euchromatic nuclei (Fig. 3). Ovaries from fry treated with 2 mg/li zinc show connective tissue growth which outpaces the formation and development of eggs (Fig. 4). The number of mature oocytes was decreased by 44%, maturing oocytes by 40%, while oogonia showed no significant decrease in the average number in transverse section counts (Fig. 5). A highly irregular egg surface morphology is exhibited (Fig. 6). The membrana propia folliculi detaches from the cortical layer, and the thecal and granulosa follicle cells contain irregularly-shaped heterochromatic nuclei (Fig. 7). The egg cortical region is highly disorganized (Fig. 8). Exposure to sublethal concentrations of zinc at 5 mg/li, resulted to extensive damage to the developing eggs; the cytoplasm show darkening indicative of changes in its normal biochemistry. Egg surfaces are ruffled. Nuclei are pyknotic (Fig. 9). No organized structure can be observed in the electron micrographs.

Zinc is a naturally-occurring heavy metal. Trace quantities of zinc is vital to biological processes as cofactors, such as in carboxypeptidases. However, studies indicate that higher concentrations can damage essential life processes (4). Toxicity is related to water hardness and synergism or antagonism of other substances in the water. Zinc toxicity to fishes at different stages of development ranges from 0.87 to 33.0 mg/l after 96-hour exposure. The probable safe concentration for most fishes is equal to or less than 0.05 mg/l (1). For *Tilapia nilotica* fry LD_{50} was set at 10 mg/li.

Tilapia nilotica Linn is a dominant freshwater teleost with high metabolic rate. In their natural habitat, eggs and embryos have some protection from pollutants by mouth brooding. It is the larval stage that is exposed to pollutants. In their gonadal development, the presence of primordial germ cells in the dorsal root of the developing mesentery, in the mesoderm ventral to the gut and in the gut endoderm right after hatching have been sequentially observed and reported (5). Oogenesis occurs earlier than spermatogenesis. Oogenesis occurs in fry of age fifty-six days (5), (6).

Organisms appear to respond in a limited number of ways to a broad range of applied stressors, a phenomenon called "general adaptation syndrome" (7). Sublethal levels of copper, cadmium, lead, and pesticides have strong inhibitory effects on gamete production and maturation. Several studies indicated that formation of gametes is either arrested or delayed.

Reproduction was the single most sensitive response in fish exposed to chronic and partial chronic tests to zinc, cadmium, copper, lead, cyanide, Guthion, Mirex, and diazanone; all these test pollutants inhibited gonadal development (8). In the case of zinc, the next most sensitive response was survival and growth in the fathead minnow (9) while growth was next to reproduction in the guppy (10).

In the same critical review (8), reproduction, evaluated through the number of eggs, was significantly reduced. After long exposure (20 days) of adult *Lebistes reticulatus* to zinc, the number of mature oocytes was significantly reduced while no significant decrease in oogonial number was noted (11). Zinc, together with copper and lead, induced significant atresia in the ovary of *Puntius chonchonius* (12). Zinc level as low as 0.035 mg/l almost completely eliminated egg production in *Pimephales promelas* (9). An inevitable consequence of the significant decrease in the eggs formed is reduction in fecundity and this in turn will reduce the survival potential of the species in contaminated ecosystems.

Follicle cells are responsible for the nourishment of the developing eggs and are the primary locus of hormone action (13). Alteration in their structural properties signifies disturbance in their functions. Heterochromatism indicates slackening in nuclear activity and this condition may be a stress symptom. This was observed in the egg cells of the fishes reared in sublethal concentrations of zinc. Nuclei with highly contorted shapes were exhibited. Damage on the egg membrane layers may account for the highly irregular morphology of egg cells in the zinc-treated group. Adverse alteration in the egg membrane may result in reduction of gas diffusion, and changes in osmoregulatory capacity. Such eggs may not be fertile or the progeny developed from such egg cells may be abnormal (2). The cortical layer plays an important role in regulating the exchange taking place between the egg cell and its surroundings. Eggs of zinc-treated fish had disorganized cortical layers. Impaired nourishment and other physiological reactions may account for the fibrous degeneration of the ovary exposed to 5 mg/l concentration of zinc. In a study undertaken to determine the concentration of heavy metals: zinc, copper, cadmium, and lead in the cyprinid *Barbus grypus* and *Barbus belaywin*, bioaccumulation of zinc was observed in the gonads, the organ observed with the highest concentration of the heavy metal, demonstrating the propensity of zinc for egg cells (14).

Hyperemia was observed in the treated ovary (Fig. 10). In this condition, blood cells appear to have rushed into the tissue, indicative of stress. Hyperemia in zinc-treated *Puntius chonchonius* testis was due to dilation in the testicular blood capillaries (13).

These findings suggest that zinc is a pollutant which impairs reproductive success and survival potential of *Tilapia nilotica* in zinc-contaminated ecosystems.

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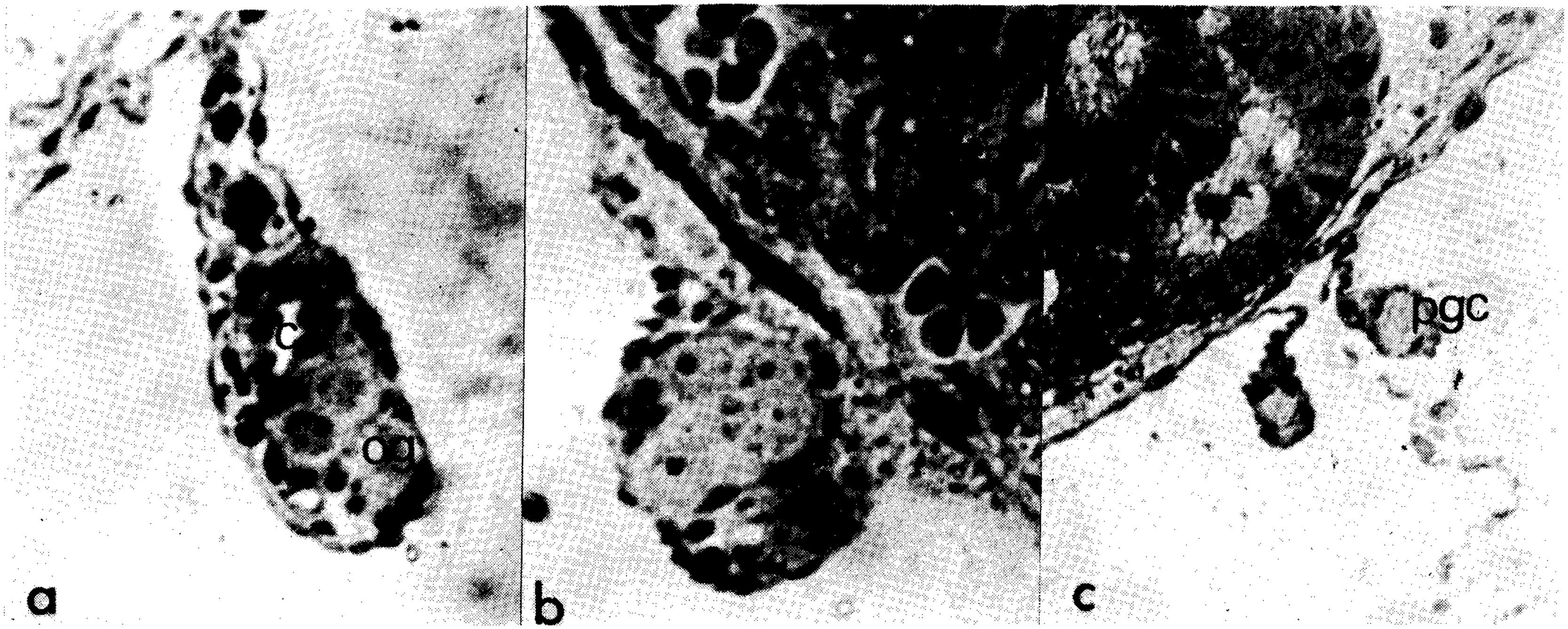


Fig. 1. Transverse section of gonads of fry at 30 days posthatching. (a) control, showing differentiated ovary with the cavity, c and some oogonia, og; (b) exposed to 2ppm zinc, still undifferentiated, large primordial germ cells, PGCs can be seen (c) exposed to 5 ppm zinc, undifferentiated gonad, much smaller with less number of PGCs (x 450).

Fig. 2. Transverse section of control ovary at 57 days posthatching, showing Type I and Type II oocytes (x 450).

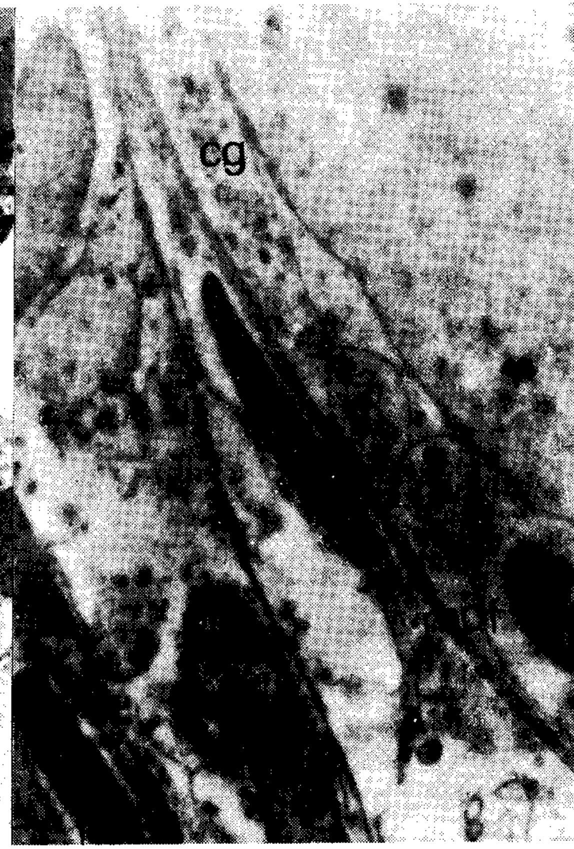
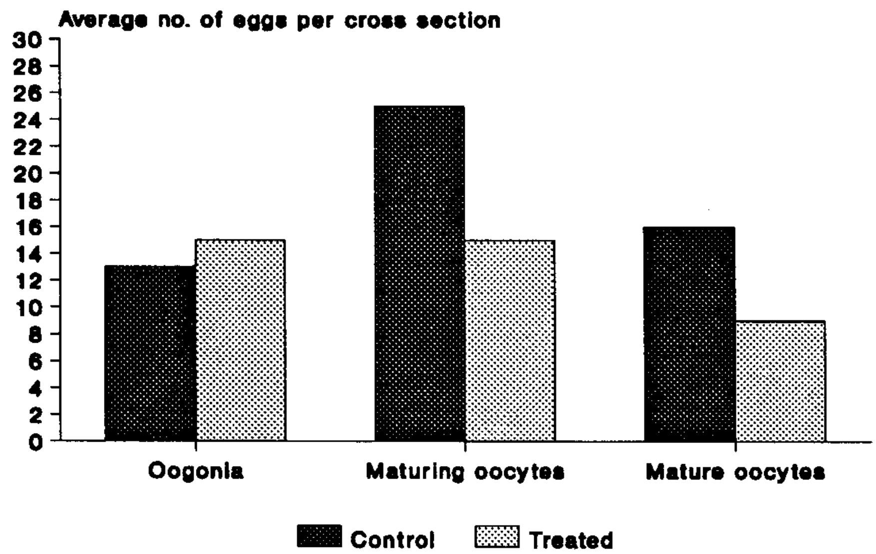


Fig. 3. Electron micrograph of control ovary at 57 days posthatching; membrane layers are organized (x 600) tfc, thecal follicle cells; cg, cortical granules; mpf, membrana propia folliculi; gfc, granulosa follicle cells.

Fig. 4. Transverse section of ovary at 57 days posthatching exposed to 2 ppm zinc showing extensive connective tissue, ct growth outpacing the formation of egg cells (x 450).



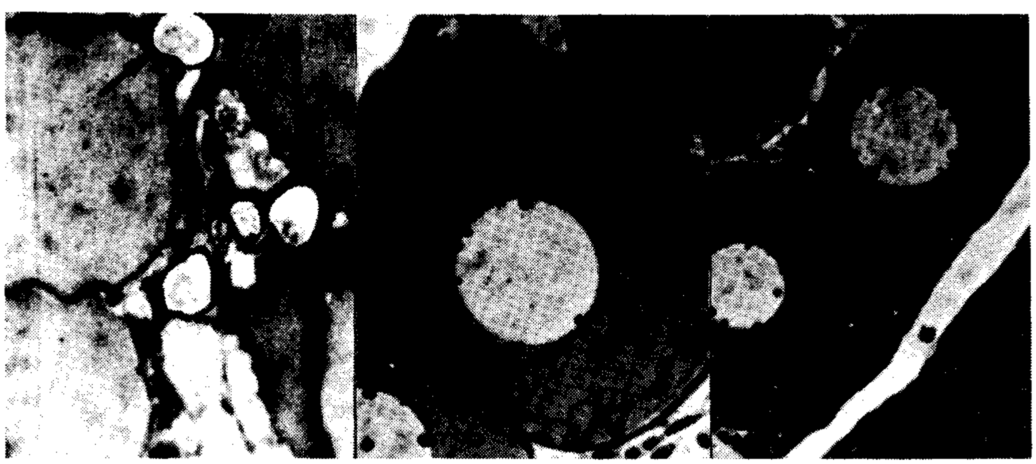
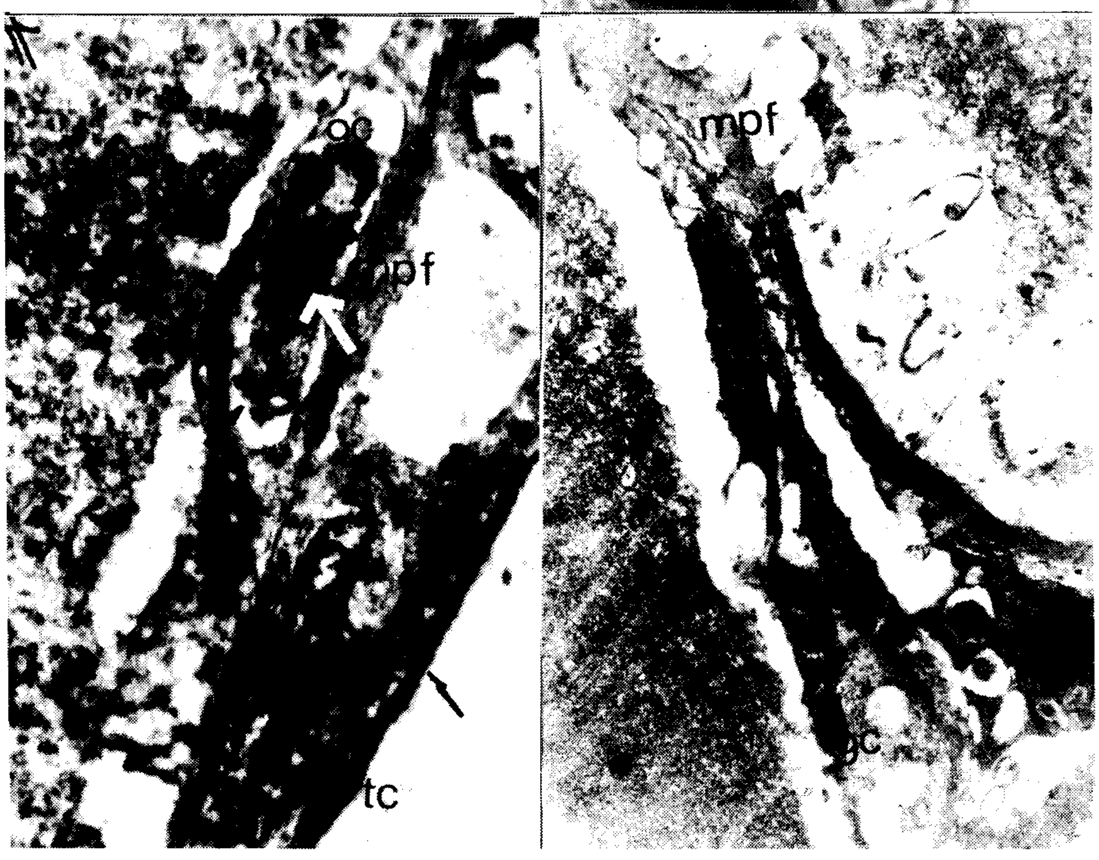


Fig. 5. Average transverse section counts of oogonia, maturing oocytes (30-103 um diameter). Ten ovaries from the control and treated group were randomly sampled then sectioned for counting.

Fig. 6. Transverse section of ovary at 57 days posthatching exposed to 2ppm zinc showing highly irregular outline of egg surface (x 450).

Fig. 7. Electron micrograph of egg membrane layers of ovary exposed to 2 ppm zinc. The membrana propia folliculi (mpf) is in the process of degeneration and is detached from the egg cortical layer. Heterochromatic nuclei of follicle cells are seen (arrows) (x 6000) tc, thecal cell; gc, granulosa cell.



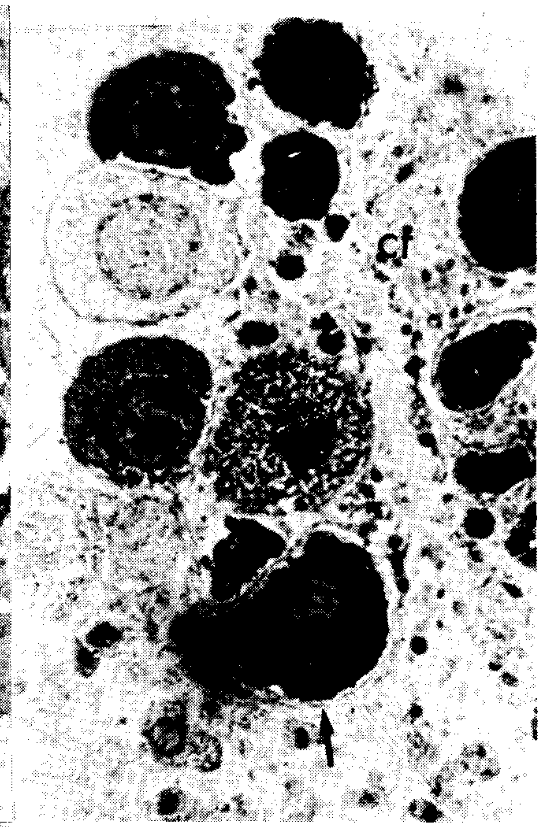


Fig. 8 (Above left). *Electron micrograph of cortical region of egg exposed to 2 ppm zinc showing disorganization, pyknotic nucleus of thecal cell (arrow) (x 6000).*

Fig. 9 (Above right). *Transverse section of ovary at 57 days posthatching, exposed to 5 ppm zinc showing degenerating connective tissue, ct and egg cells with ruffled surface (arrow) and nuclear clumps (x 450).*

Fig. 10 (Left). *Transverse section of ovary at 57 days posthatching exposed to 2 ppm zinc showing hyperemia (x 150).*