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How much are metals for next-generation clean technologies harmful to aquatic animal health? A study with cobalt and nickel effects in zebrafish (*Danio rerio*)

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ABSTRACT

Cobalt (Co) and nickel (Ni) are key metals for next-generation clean technologies with broad applications in industrial, military and commercial products. However, the knowledge about their environmental impact and toxicity to aquatic organisms remain limited, especially for the early developmental stages of fish. Thus, the current study aimed to evaluate the developmental toxicity of Co and Ni in zebrafish (*Danio rerio*). Zebrafish embryo-larval toxicity test (ZELT) was conducted with Co and Ni at different concentrations (10, 20, 40, 60, 80 and 100 mg L^{-1}) during 144 h in a static condition. Multiple biomarker responses were analyzed, such as mortality, hatching rate, neurotoxicity, cardiotoxicity, teratogenesis, and morphometric changes. Results showed high embryotoxicity of Ni compared to Co. Both metals inhibited the hatching process and induced morphological changes, such as inhibition of swim bladder inflation, yolk sac edema and pericardial edema. Co induced bradycardia in zebrafish embryos, while Ni caused tachycardia, indicating differential cardiotoxic effects. Overall, the exposure to Co and Ni disrupts early development of zebrafish, confirming their risk to the health of freshwater fish. Results indicated the ZELT as a suitable approach to assess the environmental risk of metals for next-generation clean technologies.

1. Introduction

Cobalt (Co) and nickel (Ni) are naturally occurring metals in the earth's crust, which are widely exploited in industrial processes because they are metals with high durability and corrosion resistance (USGS 2019a). Co is obtained as a byproduct of copper (Cu) and Ni mining and is exploited to produce parts for gas turbine engines, catalysts, batteries, alloys, steel, dyes, pigments, varnishes, inks, paint drying agents, among others (USGS 2019b). Both metals are strategic for next-generation technologies, which aim to create a low-carbon society and to reduce pollution (Sun et al., 2019). An example of these technologies is the electrification of automobiles, which use lithium-ion batteries as an energy source (Feng et al., 2022). In addition to Li, Co and Ni also constitute the batteries. The growing trade in electric automobiles should boost demand for these metals (Maroufi et al., 2020; Winjobi et al., 2022).

The increase in demand for Co and Ni consequently increases the production of waste, such as urban and industrial effluents, chemical waste, and mainly mining waste (Al-Shahrani 2014; Zhou et al., 2021). Mining activities move large volumes of materials and generate waste both in the extraction stage (considered sterile) and in the mining process (tailings), and the composition of these tailings is quite varied (Muniz et al., 2008). Environmental contamination is the problem in this scenario when residues and tailings do not receive adequate treatment and destination. In surface waters, Co and Ni have the toxic potential for aquatic biota, as these metals can accumulate in sediments and throughout the food chain (Ahlf et al., 2009; Zhang et al., 2020; Xu et al., 2022).

In the European Union, the limit for detection of Ni in the surface water is 0.034 mg L^{-1} (European Commission, 2008), while no limit for Co is established in surface waters. In Brazil, the maximum allowed concentration for Co for surface water is 0.05 mg L^{-1} for rivers class 1 and class 2 and 0.2 mg L^{-1} for rivers class 3. For Ni the limit is 0.025 mg L^{-1} for all river class (BRASIL, 2005). In the USA, no limit for these metals is established in surface waters. However, large amounts of these metals were found in the environment. In aquatic systems near to Co mining activities, up to 500 mg L^{-1} of Co was detected (Cheyns et al., 2014). Ni concentrations up to 245 mg L^{-1} were detected in the wastewater of the electroplating industry (John et al., 2016). Thus, this exploitation

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coupled with constant industry developments, consequently make man and the environment increasingly exposed to contamination by metals, such as Co and Ni (Muñoz and Costa, 2012).

The ecotoxicological impact of Co and Ni has been reported for different trophic levels, such as marine plankton and fish. The Co exposure inhibited the growth of diatoms (*Odontella mobiliensis, Coscinodiscus centralis, Skeletonema costatum*) and marine copepods (*Longipedia weberi*) (Karthikeyan et al., 2019), as well as induced oxidative stress on the brain, liver and kidney of the goldfish *Carassius auratus* (Kubrak et al., 2011). Similarly, Ni reduced the egg viability of the marine copepod *Acartia tonsa* (Zhou et al., 2016) and caused changes in the hepatic antioxidant enzymes and lipid peroxidation (LPO), as well as DNA damage in the gill cells and erythrocytes of neotropical fish *Prochilodus lineatus* (Palermo et al., 2015). Furthermore, Ni induced the sensitivity and reduced thickness of olfactory epithelium in zebrafish (*Danio rerio*) (Lazzari et al., 2019), while the Co induced total blocking of hair cells of the zebrafish lateral line (Stewart et al., 2017). On the other hand, the knowledge about the effects of both metals on early developmental stages of aquatic organisms, especially vertebrates, remains limited.

The zebrafish has become a widely used model in several research areas worldwide (Verma et al., 2022), especially in the Global South with a search history of more than two decades in Brazil (Trigueiro et al. 2020). The use of zebrafish has advantages such as rapid development, easy maintenance, low-cost, external fertilization, high fertility and transparent embryos, in addition to allows faster toxicological testing and having its genome sequenced, with approximately 70% similarity to humans (Sipes et al., 2011; Howe et al., 2013). The zebrafish embryo-larval toxicity test (ZELT) allows an ecotoxicological assessment of organism development from embryogenesis to larval phase (OECD 2013). The ZELT is recommended for toxicity assessment by European Union, Directive 86/609/ECC (European Commission, 1986), the Registration, Evaluation, authorisation, and Restriction of Chemicals (REACH) regulation of the European Union (European Commission, 2006), and Directive 2010/63/EU (European Commission, 2010).

In this context, the current study aimed to evaluate the developmental toxicity of Co and Ni in zebrafish. The hypothesis that the Ni and Co induce differential developmental toxicity on zebrafish was tested. The present study can contribute to the analysis and classification of the environmental risk of these metals, as well as establish zebrafish embryos as a model system for analysis of environmental impacts of metals for next-generation clean technologies.

2. Material and methods

2.1. Fish maintenance and embryo production

Adult wild-type zebrafish were obtained in zebrafish facilities at Institute of Tropical Pathology and Public Health (IPTSP) of Federal University of Goiás (UFG). Fish were maintained in a water recirculation system with oxygen saturation ≥ 60%, pH 6.8 – 7.2, temperature 26 ± 1 °C and photoperiod of 14:10 h (light: dark cycle) according to OECD (2013). Fish were fed four times a day with commercial food (Cardume® 36%; VB alimentos) and *Artemia salina* nauplii. The spawning was induced in breeding tanks (TECNOPAST®) with males and females in a ratio of 3:1. After spawning, viable embryos (2 – 4 hpf) were selected using a photomicroscope (Leica DM 750) associated with the LEICA ICC50 HD camera and LAS EZ software, based on Lammer et al. (2009). To perform the ZELT, reproduction viability ≥ 70%, and fertility rate ≥ 80% were evaluated (OECD 2013).

2.2. Zebrafish embryo-larval toxicity test (ZELT)

Cobalt chloride ($CoCl_2$) (CAS n. 7791-13-1; purity: ≥ 97%)) and nickel chloride ($NiCl_2$) (CAS n. 7791–20–0; purity: ≥ 97%) were purchased from NEON®. For both metals, a metal stock solution (100 mg L^{-1}) was made in reconstituted water (294.0 mg L^{-1} $CaCl_2.2H_2O$; 123.3 mg L^{-1} $MgSO_4.7H_2O$; 63.0 mg L^{-1} $NaHCO_3$; 5.5 mg L^{-1} KCl (ISO 1996). Test solutions were prepared by dilution of stock solutions in reconstituted water (ISO 1996).

Zebrafish embryos (4 up to 128 cell) were exposed separately to cobalt chloride ($CoCl_2$) and nickel chloride ($NiCl_2$) at different concentrations (10, 20, 40, 60, 80 and 100 mg L^{-1}) during 144 h. Similar environmental concentrations were observed in aquatic systems near to mining activities (Cheyns et al., 2014; John et al., 2016). In addition, embryos were also maintained in reconstituted water (negative control) and exposed to 3,4-dichloroaniline at 6 mg L^{-1} (positive control) (OECD 2013). Embryos were individually exposed in 24 well plates (KASVI®) containing 2 mL of the solutions (1 embryo *per* well; 30 embryos *per* experimental group) under static conditions (without renewal of the medium). The exposure was performed in triplicate under controlled temperature (26 ± 0.5 ºC), humidity (60%) and photoperiod (14/10 h light/dark) in B.O.D. incubator (Solab® SL-224/120). Similar physical and chemical parameters were observed between experimental groups and the control group (see supplementary material Table S1).

2.2.1. Multiple biomarkers assessment

During the exposure period (144 h), multiple biomarker responses were analyzed daily using a photomicroscope (Leica DM 750) associated with the LEICA ICC50 HD camera and LAS EZ software. The survival rate (%) was determined daily (24 – 144 h) using the following equations: survival rate (%) = (number of viable embryo/larvae / total number of embryo/larvae) *x* 100. Embryos with no spontaneous contraction or heart, lack of somite formation, non-detachment of the tail or coagulated embryos were considered as dead (Lammer et al., 2009; OECD, 2013). Similarly, the hatching rate (%) was determined daily (24 – 144 h) using the following equation: hatching rate (%) = (number of hatched larvae / total number of embryo/larvae) x 100 (OECD, 2013). Furthermore, the frequency (%) of several morphological alterations, such as pericardial edema, swimming bladder deformity, blood accumulation and yolk sac edema, was determined daily (24 – 144 h) according to Lammer et al. (2009) and Beekhuijzen et al. (2015). The total morphological alteration frequency is the sum of all morphological alterations.

The neurotoxicity was analyzed after 24 h of exposure by measuring the spontaneous contraction frequency (SCF) (number of movements min^{-1}) (Weichert et al., 2017). SCF has been indicated as an appropriate sublethal end point for the potential neurotoxicity of substances (Weichert et al., 2017; Krzykwa et al., 2019). Cardiotoxicity was assessed by measuring the heart rate (number of beats min^{-1}) after 48 h of exposure, especially due to the reduced movement and transparency of the embryos (Beekhuijzen et al., 2015; Babić et al., 2017). Both parameters were analyzed during 60 s in 30 embryos per experimental condition using a photomicroscope (Leica DM 750) associated with the LEICA ICC50 HD camera and LAS EZ software. For this, the embryos were positioned horizontally on a microscope slide in a thin layer of reconstituted water (ISO, 1996).

At the end of exposure period (144 h), the larvae were fixed by immersion in 4% paraformaldehyde pH 7.2 for 4 h, washed in 0.2 M PBS buffer pH 7.2 and then stored in 70% alcohol at 4 °C. Subsequently, for morphometric analysis, images were taken of the lateral and dorsal region of the zebra fish larvae ($n = 10$ *per* experimental group) using a stereomicroscope Zeiss Stemi505® coupled to the image capture system (Camera, Axiocam 105 color) and software ZEN 2.6 Blue edition ZEISS. The morphometric parameters were divided into four categories according to Malafaia et al. (2020): (i) sensory (ocular area - AO, minimum interocular distance - IDmin and maximum interocular distance - IDmax); (ii) physiological (heart area - HA, swimming bladder area - SBA and yolk sac area - YSA); (iii) structural / skeletal (head height - HH, head width - HW, head depth - HD, anus to mouth distance - AMD); (iv) structural / muscular (angle between myosepts – ABM and distance between myosepts - DBM) (see supplementary material Figure S1). The

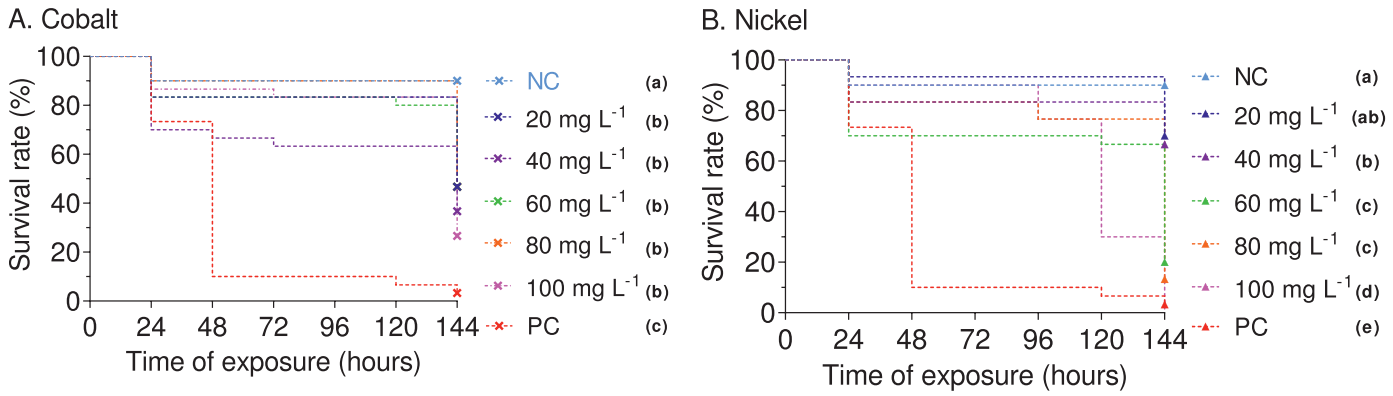

Fig. 1. Survival rate (%) of zebrafish embryos and larvae from a control group and exposed to cobalt (A) and nickel (B) at different concentrations (20, 40, 60, 80 e 100 mg L^{-1}) during 144 h. NC: Negative control; PC: Positive control. Letters indicate statistical differences ($p < 0.05$), while similar letter show no statistically significant differences ($p > 0.05$).

measurements of each parameter were performed using the Image J software and results expressed as mean ± standard deviation.

2.5. Statistical analysis

Statistical analyses were performed using the software R v. 4.0.2 (R Core Team 2020) and graphs were organized in the Prism 7 GraphPad® software. Normality and homogeneity of variances were evaluated through the residual dispersion graphics and the Levene test, respectively. Log rank test were performed to analyze the survival and the hatching rates (survfit function), followed by multiple comparisons using the Pairwise survdiff test. Generalized linear models (GLM) were performed to assess differences between exposed groups to spontaneous contraction frequency (SCF) and heart rate, using the log link function to the Poisson family (for heart rate) and the Negative Binomial family (for SCF, due to overdispersion). For morphological changes, a Zero-inflated count model (ZIP) with poisson distribution was fitted due to excess zeros (*'pscl'* package). Multiple comparisons tests for the fitted models were performed using the *'emmeans'* package. The zebrafish morphometric measures from the different exposed groups were analyzed using the Kruskal-Wallis test followed by the Dunn's *post-hoc* test for pairwise comparisons of the ranked data. Results were considered significant when $p < 0.05$. Due to the delay in the development of zebrafish embryos exposed to the positive control, this experimental group was not included in the statistical analysis of SCF, heart rate and morphometric parameters.

3. Results and discussion

The differential developmental toxicity of Co and Ni in zebrafish (*Danio rerio*) was analyzed by multiple biomarker responses. Overall, both metals for next-generation clean technologies can induce toxic effects in zebrafish embryos and larvae according to the concentration and exposure time, as described below.

3.1. Survival and hatching rates

Results showed differential survival rate of zebrafish after exposure to Co and Ni during 144 h (Fig. 1A and 1B). Both metals decreased the survival rate during the exposure period when compared to the control group. The survival rate of the negative control group was 90%, as recommended by OECD (2013). However, the zebrafish exposed to Co showed a decline in survival rate over time, which was lower than 50% after exposure to 20, 40, 60, 80 and 100 mg L^{-1} ($\chi^2_{(6,\ 210)} = 89.70$, $p < 0.001$; Fig. 1A). Embryos exposed to Ni showed higher mortality when compared with those exposed to Co ($\chi^2_{(6,\ 210)} = 138.00$, $p < 0.001$). The exposure to Ni affected survival in a concentration-dependent pattern and it was more lethal above 60 mg L^{-1}, with zebrafish survival rates below 20% after exposure to 60, 80 and 100 mg L^{-1} (Fig. 1B). These results confirmed that the embryotoxic effects of both metals were time and concentration dependent. Cai et al. (2012) also reported embryotoxic effects in zebrafish induced by $CoCl_2$ at similar concentrations (20 to 100 mg L^{-1}). Kienle et al. (2008, 2009) showed mortality of zebrafish exposed to lower $NiCl_2$ concentrations (10 and 15 mg L^{-1}) but using different exposure conditions (glass Petri plates; 30 embryos per plate under semi-static conditions). Thus, results showed that the Co toxicity to early developmental stages of zebrafish depends on experimental design.

Similar to survival rate, both metals reduced the hatching rate of zebrafish when compared to the control group in a time and concentration-dependent pattern (Fig. 2A and 2B). After 144 h, embryos exposed to Co showed a reduced hatch rate ($\chi^2_{(6,\ 210)} = 81.60$, $p < 0.001$), even at the lowest concentrations (Fig. 2A). Ni-exposed embryos also showed a significant reduction in hatching rates ($\chi^2_{(6,\ 210)} = 136.00$, $p < 0.001$), mainly at concentrations above 60 mg L^{-1}, that were similar to the positive control (Fig. 2B). Relatively, Co exposure to 20 and 40 mg L^{-1} inhibited 51.5 and 66.3%, respectively, when Ni exposure at the same concentrations decreased 27.8 and 30.4% of hatching rate, respectively. On the other hand, the unexposed embryos showed a hatching rate of 90% after 72 h, as recommended by Lammer et al. (2009) and (OECD 2013). Hatching delay in zebrafish embryos exposed to Ni was also reported by Kienle et al. (2008), Scheil and Köhler (2009), Scheil et al. (2009) and Nabinger et al. (2018). Regarding exposure to Co, the literature lacks studies that discuss delays in the hatching process due to Co toxicity. The present study indicated that the Co exposure at a concentration above 20 mg L^{-1} could inhibit the zebrafish hatching process.

The zebrafish hatching is a complex process involving enzyme activities and the spontaneous contraction of embryos (Hagenmaier 1974; Sreedevi et al., 2014). During embryonic development, metal ions may enter the egg, causing changes in chorion permeability. Metals can interfere at the production of the chorionase enzyme, which is responsible for the chorion disintegration during hatching. Inhibition of this enzyme may result in delayed hatching process (Jezierska et al., 2009). The hatching delay induced by several metals, such as zinc (Zn), manganese (Mn), copper (Cu) (Wu et al., 2014), cadmium (Cd) (Capriello et al., 2019) and lead (Pb) (Zhao et al., 2020) can reduce the survival, reproductive capacity and the fish population. In addition, the late hatching may make animals more vulnerable to predation due to decreasing larvae activity (Kienle et al., 2009).

3.2. Neurotoxicity

The spontaneous contraction frequency (SCF) (number of movements min^{-1}) of zebrafish embryos exposed to Co was different between

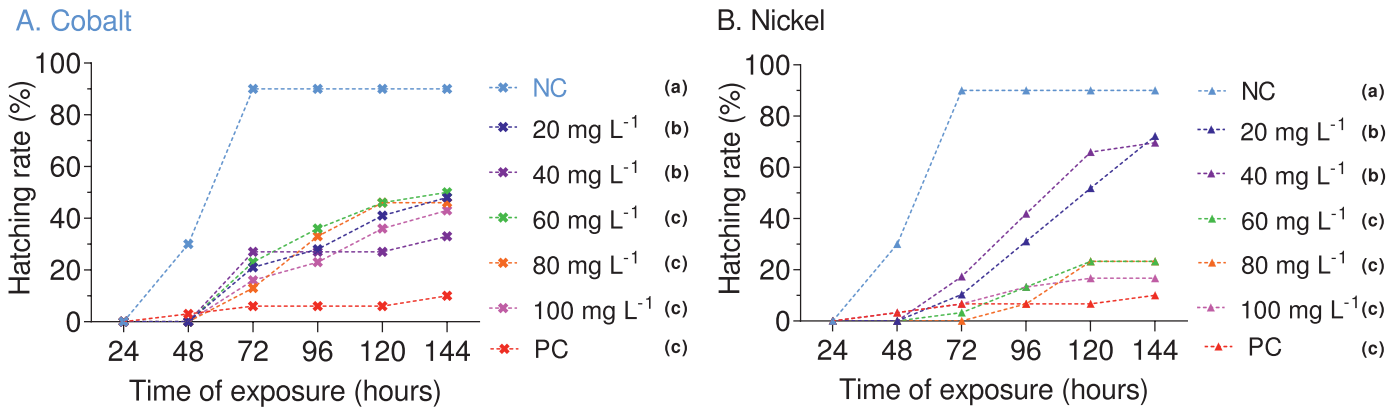

Fig. 2. Hatching rate (%) of zebrafish from a control group and exposed to cobalt (A) and nickel (B) at different concentrations (20, 40, 60, 80 and 100 mg L^{-1}) during 144 h. NC: Negative control; PC: Positive control. Letters indicate statistical differences ($p < 0.05$), while similar letter show no statistically significant differences ($p > 0.05$).

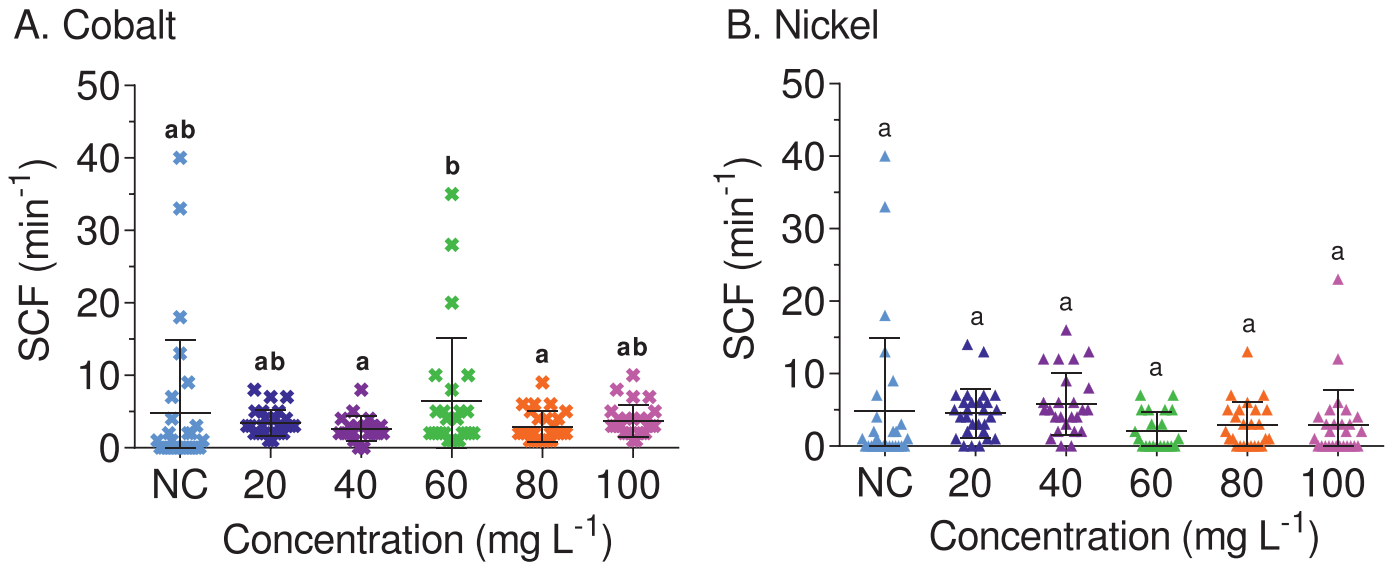

Fig. 3. Spontaneous contraction frequency – SCF (number min^{-1}) in zebrafish embryos from control group and exposed to cobalt (A) and nickel (B) at different concentrations (20, 40, 60, 80 and 100 mg L^{-1}) for 24 h. NC: Negative control; PC: Positive control. Letters indicate statistical differences ($p < 0.05$), while similar letter show no statistically significant differences ($p > 0.05$).

40, 60 and 80 mg L^{-1} concentrations ($\chi^2_{(5, 180)} = 14.82$, $p = 0.011$) (Fig. 3A), while for Ni-exposure embryos the SCF was similar to the negative control ($\chi^2_{(5, 180)} = 14.82$, $p = 0.061$) (Fig. 3B), indicating that the Co showed neurotoxic effects when compared to Ni, at the same exposure conditions. Although the neurotoxicity mechanism of Co in fish is not well described in the literature, this metal induced neuronal necrosis and increased hippocampus lipid peroxidation in Wistar rats exposed to 4 mg kg^{-1} by intraperitoneal injection (Zheng et al., 2019), as well as blocked the neuromasts of the lateral line in zebrafish exposed to 5 mM (Stewart et al., 2017). Also, Sonnack et al. (2015) observed that zebrafish embryos exposed to $CoSO_4$ had abnormal primary and secondary motor neurons development and neuromast cells damage. Subsequently, these same authors showed that neurotoxicity might be associated with downregulated NK2 homeobox 2a (nkx2.2a) and Claudin b (cldnb) genes, involved in the nervous system and neuromasts development, respectively, after exposure to Co at 0.5 and 1.4 mg L^{-1} (Sonnack et al., 2017). Krzykwa et al. (2019) evaluate the responsiveness of zebrafish embryos to lower Ni concentrations (< 2 mg L^{-1}) and did not found significant differences in SCF. Instead, an acute test with Ni on 5 day old zebrafish larvae showed decreased on the locomotory activity at 10 and 20 mg L^{-1} (Kienle et al., 2008). Ni changed locomotory activity, inhibited antioxidant enzymes, and induced apoptosis in fish (Zheng et al., 2014) and its toxic effects were associated with oxidative damages by ROS production. Topal et al. (2015) exposed rainbow trout (*Oncorhynchus mykiss*) adults to Ni and they confirmed histopathological damages in brain tissues by demyelination of the fish cerebellum and a significant decrease in brain acetylcholinesterase (AChE) enzyme activities. AChE is very important to fishes for many physiological functions, such as locating food and escaping predators. Decreased AChE enzyme has been linked to essential functions, such as feeding and swimming, which can reduce fish fitness (Glusczak et al., 2006).

3.4. Cardiotoxicity

The heart rate of zebrafish embryos exposed to both metals is presented in Fig. 4A and 4B After 48 h of exposure, embryos exposed to Co at all concentrations presented reduced heart rate (bradycardia) when compared to control group ($(\chi^2_{(5, 180)} = 93.96$, $p < 0.001)$; Fig. 4A), Similarly, cases of bradycardia in zebrafish exposed to Co (0.1 and 0.5 mg L^{-1}) were also reported by Cai et al. (2012). According to previous studies, the decrease in heart rate may occur due to the inhibition of AChE by the pollutant, which may cause an increase in the synap-

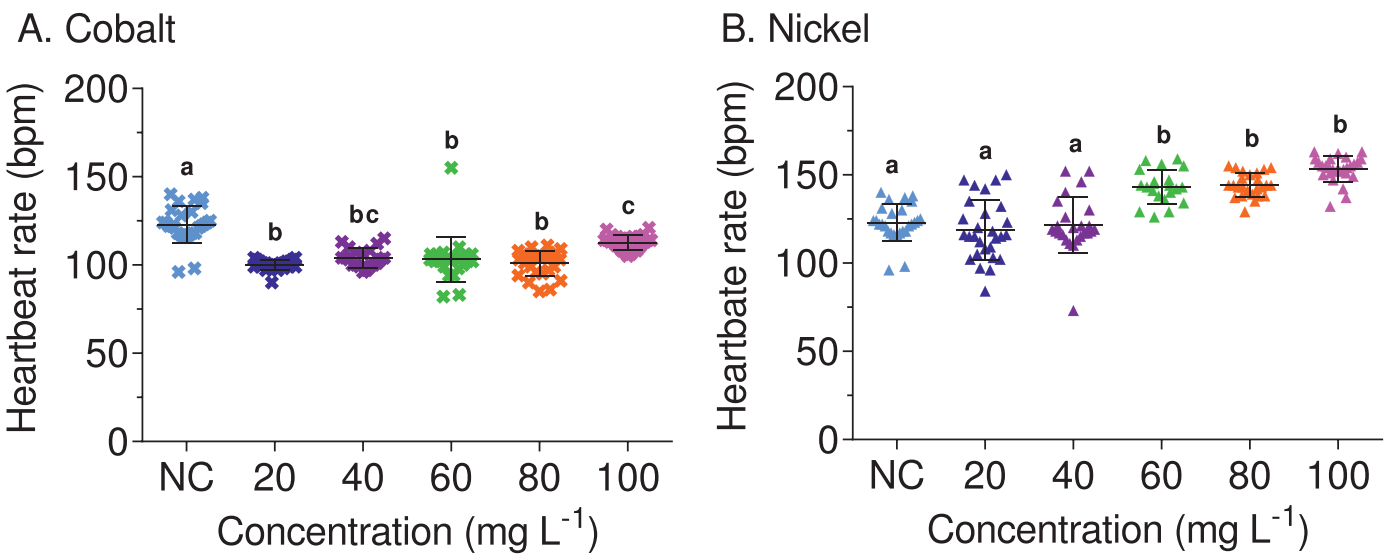

Fig. 4. Heart rate (min^{-1}) in zebrafish embryos from a control group and exposed to cobalt (A) and nickel (B) at different concentrations (20, 40, 60, 80 and 100 mg L^{-1}) for 48 h. NC: Negative control; PC: Positive control. Letters indicate statistical differences ($p < 0.05$), while similar letter show no statistically significant differences ($p > 0.05$).

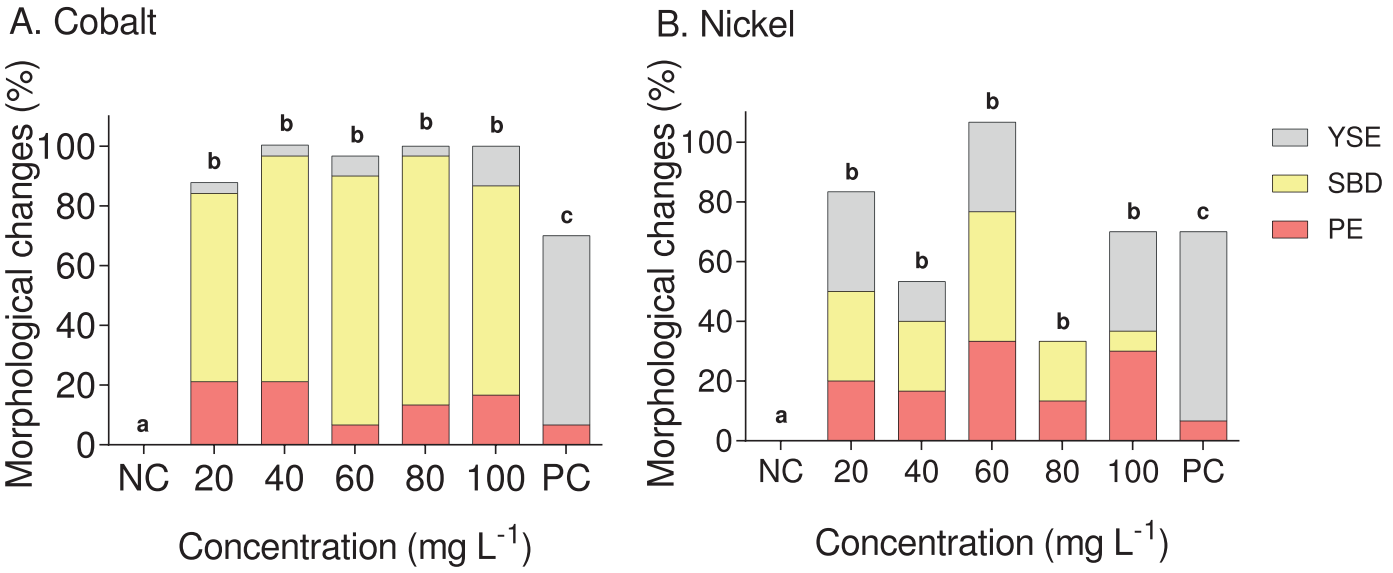

Fig. 5. Morphological changes frequency (%) in zebrafish from a control and exposed to cobalt (A) and nickel (B) after 144 h of exposure. NC: Negative control; PC: Positive control; YSE: Yolk Sac Edema; SBD: Swim Bladder Deformity; PE: Pericardial Edema. Letters indicate statistical differences ($p < 0.05$), while similar letter show no statistically significant differences ($p > 0.05$).

tic concentrations of acetylcholine (ACh); consequently, cardiac muscle contractions are inhibited through interaction with muscarinic receptors on *Danio rerio* (Küster and Altenburger, 2007; Carlsson et al., 2014). Co-induced bradycardia has also been reported in Wistar rat studies. Oyagbemi et al. (2018) reported antioxidant enzymes decreasing (GPx, CAT and GST) in cardiac tissue of Wistar rats treated orally with Co at 150, 300 and 600 ppm of $CoCl_2$, indicating cardiotoxic effects mediated by ROS production and oxidative stress. They also noticed a higher Bax protein expression in the cardiac tissue, an important apoptosis regulator associated with BCL-2 protein.

In opposite to Co, the embryos exposed to Ni presented tachycardia when compared to the control group in a concentration-dependent pattern ($\chi^2_{(5,\ 180)} = 210.17, p < 0.001$). A significant difference in the heart rate was observed between control group and the zebrafish exposed to Ni at 60, 80 and 100 m L^{-1}, which presented a tachycardia rate of around 47, 63 and 83%, respectively. Individuals exposed to Ni at 20 and 40 mg L^{-1} presented significant difference compared to the 60, 80 and 100 mg L^{-1} groups. The literature does not report cases of Ni-induced tachycardia in zebrafish. However, there are reports of Ni-induced cardiotoxicity effects in other organisms such as rodents (Ilbäck et al., 1994; Lou et al., 2013), humans (Afridi et al., 2011) and other types of fish such as medaka marine (*Oryzias melastigma*) (Liu et al., 2021). Liu et al. (2021) observed tachycardia in embryos and larvae of marine Medaka (*Oryzias melastigma*) exposed to Ni at concentrations ranging from 2 to 65 mg/L. According to the authors, Ni triggered a stress response in the embryos, which caused an increase in their metabolic rate and heart rate to accelerate a detoxification process.

3.5. Morphological changes and morphometric analysis

Potential teratogenic effects of Co and Ni on zebrafish were evaluated daily during the exposure period (144 h). There were significant

differences in the number of morphological changes in zebrafish embryos and larvae exposed to both Co ($\chi^2_{(6,\ 210)} = 30.79$, $p < 0.001$) and Ni ($\chi^2_{(6,\ 210)} = 44.36$, $p < 0.001$). The frequency (%) of morphological alterations induced by Co and Ni, and their reaction patterns are in Table 1, Figs. 5 and 6. Pericardial edema, swim bladder deformity and yolk sac edema were the most frequent morphological changes in the zebrafish embryo and larvae after exposure to both metals when compared to the control group (Figs. 5-6). Swimming bladder deformity was the most frequent alteration in zebrafish larvae exposed to Co, which presented a minimum frequency of 62.96% after exposure to Co at 20 mg L^{-1} and a maximum frequency of 83.33% after exposure to Co at 60 and 80 mg L^{-1} (Fig. 5A). Besides, zebrafish larvae exposed to Co showed a higher frequency of swimming bladder deformity when compared to those exposed to Ni. The frequency of zebrafish larvae with swimming bladder deformity was a minimum of 6.67% and a maximum of 43.33% after exposure to Ni at 100 and 60 mg L^{-1}, respectively (Fig. 5B).

Studies with zebrafish report the important role of thyroid hormones in the swim bladder development. Some chemicals, when present in water, can alter the functioning of the thyroid gland, and consequently, it can inhibit the swim bladder inflation. Stinckens et al. (2016) evaluated zebrafish exposure to 2-mercaptobenzothiazole, which is a thyroid peroxidase inhibitor and observed that this chemical affects the inflation of fish swimming bladder. Another study found malformation in the swimming bladder of zebrafish embryos exposed to perfluoropolyether carboxylic acids (PFECAs), chemicals widely used in the industry. Results indicated that exposure to PFECAs reduced levels of triiodothyronine (T3) and thyroxine (T4) (thyroid hormones) in fish that did not inflate the swim bladder (Wang et al., 2020). Studies relating to toxic action of Co and Ni to damage the endocrine system of fish are absent in the literature. However, in the 1950s, Co was widely used in medical treatments because the metal is present in the composition of cyanocobalamin (vitamin B12), which acts to prevent anemia. In contrast, when metal is administered in high doses, damages to the neurological, cardiovascular and endocrine systems in humans were reported (Leyssens et al., 2017).

Dahms et al. (2014) and Garcia et al. (2020) report cases of dysregulation in the endocrine system caused by Co poisoning in patients undergoing total hip arthroplasty. After the placement of the prosthesis, the patients developed hypothyroidism, which was related to elevated levels of Co in the blood, in the order of a thousand times higher than the normal level. The high Co levels in the patients' blood were related to the type of prosthesis used. The primary materials used in hip prostheses have Co in its constitution, which can be released in the body.

In addition to endocrine disruption, studies report a relationship between heart damage and zebrafish swimming bladder inhibition (Yue et al., 2015; Mitovic et al., 2021). Yue et al. (2015) and Mitovic et al. (2021) observed cardiac damage and failure of swimming bladder inflation in zebrafish exposed to dioxin and cadmium respectively. According to the authors, changes in cardiac function may influenced the formation of the swimming bladder in zebrafish. Winata et al. (2010) showed that blood circulation plays an important role in the development of the swimming bladder in zebrafish.

Another alteration observed in zebrafish exposed to both metals was pericardial edema, which was more frequent in embryos exposed to Ni (Fig. 6). Some studies have linked cardiac damage in humans and animals exposed to metals. Charette et al. (2017) reported the development of cardiomyopathy in humans due to the toxic action of Co. Martin et al. (2015) observed heart failure in humans also exposed to Co. For Ni, studies report variations in heart rate caused by exposure to Ni in zebrafish (Nabinger et al., 2018) and in rats (Chuang et al., 2013) and arrhythmia and altered systolic function in rats (Lou et al., 2013). Although the studies showed cardiac changes caused by Co and Ni, the mechanisms related to the cardiotoxicity of metals are not fully elucidated. Some research indicates that these mechanisms involve oxidative stress due to an increase in ROS (Chuang et al., 2013; Lou et al., 2013;

Table 1
Morphological alteration frequency (%) in zebrafish induced by Co, Ni and PC and NC during 144 h of exposure. Results are expressed as mean ± standard deviation. NC: Negative control; PC: Positive control; n.o. = not observed**Groups**.

		Morphological alteration frequency (%)									
		Pericardial edema	Blood accumulation	Spinal curvature	Tail flexure	Growth retardation	Head malformation	Absence or irregular size of eyes	Swim bladder deformity	Deformities mouth	**Yolk sac edema**
Control group	**NC**	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.	n.o.
	PC	13.33 ± 5.77	n.o.	n.o.	3.33 ± 11.55	n.o.	56.67 ± 11.55	13.33 ± 11.65	n.o.	n.o.	70.00 ± 20.00
Co concentration (mg L^{-1})	**20**	21.11 ± 11.71	n.o.	n.o.	n.o.	n.o.	3.70 ± 6.42	1.75 ± 3.04	62.96 ± 22.45	n.o.	3.70 ± 6.42
	40	21.11 ± 11.71	n.o.	n.o.	n.o.	3.70 ± 6.42	n.o.	n.o.	75.56 ± 7.70	n.o.	3.70 ± 6.42
	60	6.67 ± 11.55	3.33 ± 5.77	10.00 ± 0.00	3.33 ± 5.77	n.o.	n.o.	n.o.	83.33 ± 5.77	n.o.	6.67 ± 11.55
	80	13.33 ± 15.28	n.o.	3.33 ± 5.77	n.o.	n.o	10.00 ± 17.32	n.o.	83.33 ± 11.55	3.33 ± 5.77	3.33 ± 5.77
	100	16.67 ± 20.82	n.o.	10.00 ± 17.32	6.67 ± 11.55	n.o.	n.o.	n.o.	70 ± 10	n.o.	13.33 ± 15.28
Ni concentration (mg L^{-1})	**20**	16.67 ± 20.82	10.00 ± 10.00	n.o.	3.33 ± 5.77	n.o.	6.67 ± 5.77	10.00 ± 10.00	33.33 ± 11.55	n.o.	33.33 ± 15.28
	40	13.33 ± 15.28	13.33 ± 23.09	n.o.	3.33 ± 5.77	n.o.	6.67 ± 11.55	6.67 ± 11.55	23.33 ± 20.82	n.o.	13.33 ± 15.28
	60	23.33 ± 20.82	6.67 ± 5.77	10.00 ± 0.00	n.o.	n.o.	n.o.	n.o.	43.33 ± 5.77	n.o.	30.00 ± 15.28
	80	33.33 ± 15.28	3.33 ± 5.77	3.33 ± 5.77	n.o.	n.o.	n.o.	n.o.	20.00 ± 17.32	n.o.	n.o.
	100	30.00 ± 2.00	n.o.	n.o.	n.o.	n.o.	3.33 ± 5.77	3.33 ± 5.77	3.33 ± 5.77	n.o.	33.33 ± 20.82

Results are expressed as mean ± standard deviation. NC: Negative control; PC: Positive control; n.o. = not observed.

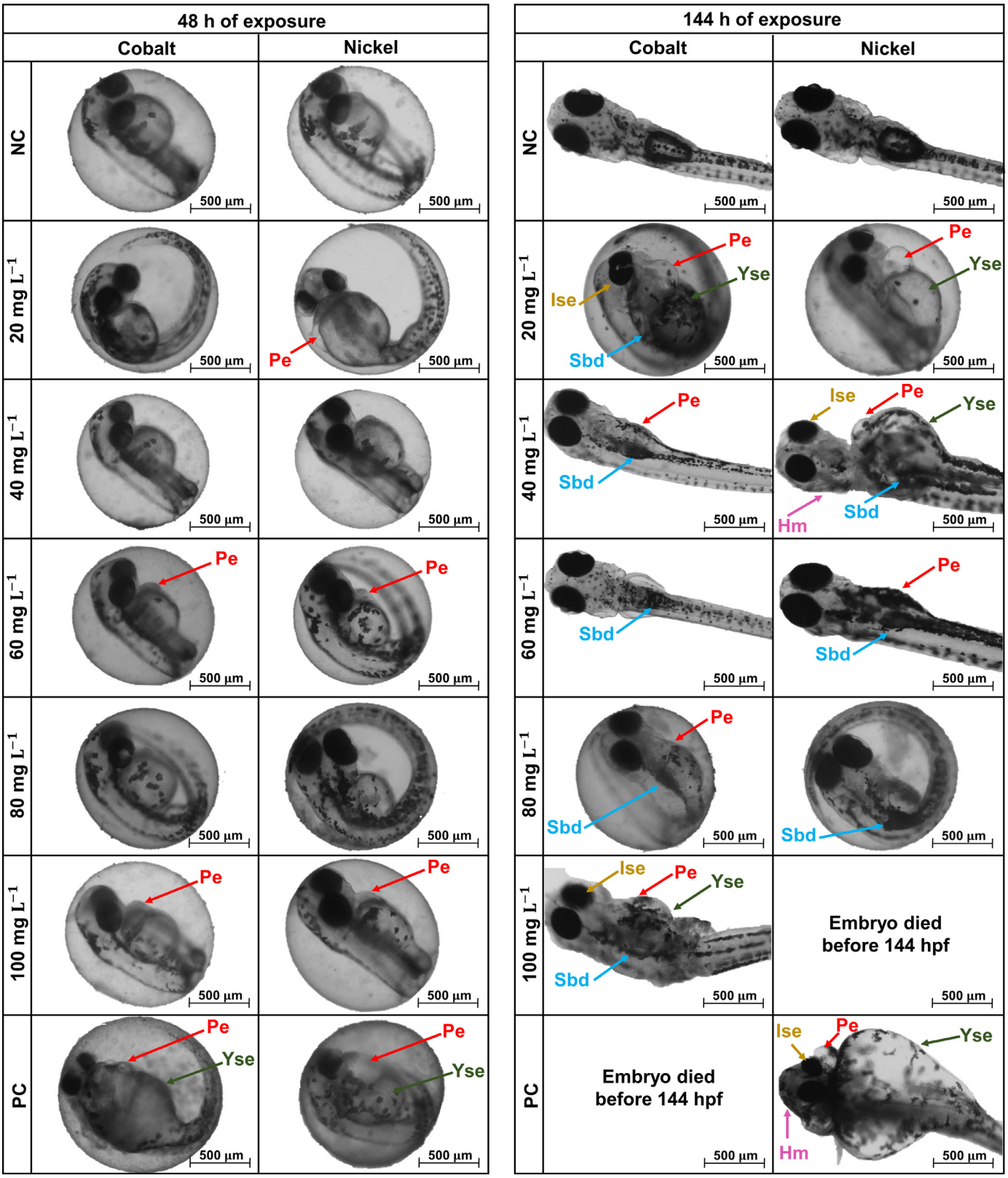

Fig. 6. Morphological changes in zebrafish embryos and larvae of the control groups and after exposure to cobalt and nickel for 48 and 144 h of exposure. NC: Negative control; PC: Positive control; Pe: pericardial edema; Yse: Yolk sac edema; Sbd: Swim bladder deformity; Ise: Irregular size of eyes; Hm: Head malformation. Bar scale = 500 µm.

Table 2
Morphometric parameters in zebrafish larvae after exposure to Co and Ni at concentrations (20, 40, 60, 80 and 100 mg L^{-1}) for 144 h.

	Category	Morphological alteration	Control	20 mg L^{-1}	40 mg L^{-1}	60 mg L^{-1}	80 mg L^{-1}	100 mg L^{-1}	$\chi 2$	p
Cobalt	**Sensory**	Ocular area	184.26 ± 9.56	161.99 ± 25.64	149.02 ± 16.74	154.11 ± 20.71	137.76 ± 15.47	154.15 ± 21.02	21.26	< 0.001
		Minimal interocular distance	6.08 ± 1.25	4.31 ±1.13	4.09 ± 0.77	2.76 ± 0.82	3.77 ± 1.09	4.09 ± 0.99	28.93	< 0.001
		Maximum interocular distance	16.83 ± 0.91	26.99 ± 3.54	27.76 ± 1.79	28.49 ± 5.43	25.67 ± 1.79	26.31 ± 0.54	27.23	< 0.001
	Physiological	Heart area	35.07 ± 2.39	22.46 ± 6.89	32.79 ± 4.39	27.38 ± 4.86	28.55 ± 5.23	29.78 ± 8.22	24.55	< 0.001
		Swimming bladder area	153.07 ± 19.62	62.65 ± 21.56	70.98 ± 22.75	58.05 ± 26.54	58.02 ± 30.76	76.11 ± 18.65	28.58	< 0.001
		Yolk sac area	379.62 ± 21.82	262.38 ± 70.46	286.59 ± 43.89	249.21 ± 50.03	299.38 ± 72.22	361.55 ± 52.03	24.59	< 0.001
	Structural skeletal	Head height	26.75 ± 1.61	23.51 ± 3.06	24.25 ± 1.18	23.41 ± 1.40	24.65 ± 1.36	25.23 ± 0.69	22.46	< 0.001
		Head width	25.22 ± 0.97	23.04 ± 3.09	25.29 ± 2.18	22.29 ± 2.57	23.11 ± 1.50	23.61 ± 0.96	18.11	.003
		Head depth	38.53 ± 1.16	32.29 ± 6.40	35.83 ± 2.32	33.017 ± 1.89	32.48 ± 2.89	31.46 ± 1.13	30.89	< 0.001
		Anus to Mouth Distance	105.23 ± 3.93	93.51 ± 8.59	98.05 ± 7.49	90.931 ± 6.73	93.49 ± 6.46	92.57 ± 5.21	22.45	< 0.001
	Sstructural muscle	Angle between myosepts	95.29 ± 3.66	94.11 ± 6.58	102.07 ± 1.45	98.515 ± 6.52	98.28 ± 7.3	103.24 ± 3.51	18.44	.002
		Distance between myosepts	3.89 ± 0.28	3.27 ± 0.34	3.61 ± 0.23	2.882 ± 0.29	3.435 ± 0.11	3.06 ± 0.41	32.45	< 0.001
Nickel	**Sensory**	Ocular area	184.26 ± 9.56	163.26 ± 16.76	183.48 ± 16.11	168.99 ± 22.83	179.62 ± 15.15	n.a	9.57	.049
		Minimal interocular distance	6.08 ±1.25	2.81 ± 0.37	6.25 ± 1.41	5.48 ± 0.51	8.41 ± 2.98	n.a	24.78	< 0.001
		Maximum interocular distance	16.83 ± 0.91	13.91 ± 0.75	28.86 ± 1.90	29.17 ± 1.65	31.99 ± 4.95	n.a	33.22	< 0.001
	Physiological	Heart area	35.07 ± 2.39	31.86 ± 5.08	32.75 ± 4.61	40.28 ± 7.63	32.14 ± 7.48	n.a	5.99	.200
		Swimming bladder area	153.07 ± 19.62	119.62 ± 33.43	125.60 ± 8.22	86.92 ± 40.75	95.25 ± 19.24	n.a	20.24	< 0.001
		Yolk sac area	379.62 ± 21.82	333.65 ± 28.67	348.12 ± 31.19	364.41 ± 78.83	356.57 ± 35.14	n.a	8.57	.073
	Structural skeletal	Head height	26.75 ± 1.61	3.88 ± 0.18	26.67 ± 1.06	25.12 ± 1.74	26.58 ± 2.19	n.a	24.55	< 0.001
		Head width	25.22 ± 0.97	12.58 ± 0.44	25.11 ± 0.99	25.31 ± 0.96	27.00 ± 4.10	n.a	22.09	< 0.001
		Head depth	38.53 ± 1.16	17.24 ± 1.42	37.98 ± 1.43	37.37 ± 1.52	36.97 ± 2.73	n.a	23.3	< 0.001
		Anus to Mouth Distance	105.23 ± 3.93	97.63 ± 6.64	99.21 ± 4.29	97.88 ± 8.01	94.98 ± 1.59	n.a	14.07	.007
	Sstructural muscle	Angle between myosepts	95.29 ± 3.66	100.80 ± 6.06	98.07 ± 4.09	97.71 ± 3.77	105.83 ± 3.05	n.a	10.9	.028
		Distance between myosepts	3.89 ± 0.28	3.36 ± 0.23	3.52 ± 0.18	3.26 ± 0.13	3.42 ± 0.25	n.a	20.11	< 0.001

Kruskal-Wallis results to Co (df = 5, $n = 57$) and Ni (df = 4, $n = 40$) exposures. n.a: morphometry not analyzed in organisms exposed to 100 mg/L of nickel. Mortality at this concentration was 100%.

Nabinger et al., 2018), which can cause oxidative damage to lipids, DNA and proteins, and consequently cause cell death (Circu and Aw 2010; Mugoni et al., 2014).

In addition to deformation in the swimming bladder and edema in the pericardium, fish exposed to Co and Ni also showed yolk sac edema, which was more frequent in exposure to Ni (Fig. 5). Yolk sac edema in zebrafish larvae has been associated with changes in the glomerular filtration barrier functions (Hanke et al., 2013). Based on observed results, the potential effect of both metals in the integrity of the glomerular filtration barrier of fish deserver further analysis. Furthermore, morphometric parameters of zebrafish larvae exposed to Co and Ni at 20, 40, 60, 80 and 100 mg L^{-1} for 144 h are shown in the Table 2. The exposure of zebrafish larvae to Co caused a significant reduction in the swimming bladder of fish in all studied concentrations. As for exposure to Ni, this reduction was significant only for concentrations of 60 mg L^{-1} and 80 mg L^{-1}, confirming the differential developmental toxicity induced by metals for next-generation clean technologies.

4. Conclusions

The present study showed that Co and Ni induce a toxic effect in zebrafish embryos and larvae, confirming the initial hypothesis. Ni was more embryotoxic metal than Co. Both metals inhibited the hatching process in a concentration-dependent pattern. Ni and Co caused changes in heart rate and malformations in the pericardium, yolk sac, and swimming bladder of exposed zebrafish. The most frequent morphological change was deformity of the swimming bladder of fish exposed to Co, indicating Co affects the endocrine system of zebrafish. This study highlights the importance of assessing the impacts of Co and Ni on aquatic biota, as these metals are constantly exploited in industrial activities. Also, it was observed that the effects of these metals on zebrafish are not yet fully known and explained in the literature. More research must be carried out to increase knowledge about the mechanisms of action and toxicity of Ni and Co in fish species. Zebrafish was a suitable model system to assess the toxicity of metals for next-generation clean technologies, such as Ci and Ni, in aquatic organisms.

Declaration of Competing Interests

The authors declare no conflict of interest.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.hazadv.2022.100160.

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