

The toxicity of nickel and the effects of sublethal levels on haematological parameters and behaviour of the fish, *Oreochromis niloticus*

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ABSTRACT

Specimens of *Oreochromis niloticus* were exposed to different concentrations of nickel chloride. Lc-50 at 96 hours was computed at 27.2 mg/l. Remarkable changes in the behaviour of fish exposed to lethal (26.0 to 28.5 mg/l) and sublethal (1.5, 3.0 and 5.0 mg/l) concentrations were observed. Significant ($P < 0.01$) changes in respiratory behaviour (cough and yawns) were registered in fish exposed to sublethal concentrations. Aggressive behaviour (nudge and nip) and stress—related discomfort movements (fin flickering, jerking movement and swimming) were also increased in exposed fish. A positive dose—response relationship was demonstrated for these changes in behaviour. Significant ($P < 0.01$) changes in haematological parameters such as a decrease in total leucocyte count and an increase in erythrocyte count, haemoglobin concentration and haematocrit were also recorded. Differential leucocyte counts showed a significant ($P < 0.01$) fall in the number of thrombocytes and lymphocytes and an increase in granulocytes (neutrophils and eosinophils) and monocytes.

MCV, MCH and MCHC values were also affected but the changes were insignificant ($P > 0.01$).

INTRODUCTION

The increasing use of heavy metals in industry in the modern world adversely affects the aquatic environment because most industrial wastes are discharged into water (Alabaster & Lloyd, 1982). This poses a great danger to aquatic organisms including fishes. In particular, the increasing use of nickel compounds in electroplating, polishing and paint pigment industries has aroused special concerns and stressed the need for understanding the mechanisms of its toxicity. Several investigations have been made to assess the acute and chronic toxicity of nickel compounds to fish and other animals. Rehwoldt *et al.* (1971), Pickering (1974), Hale (1977), Khangarot (1981), Taylor *et al.* (1985) have studied the acute toxicity of nickel compounds to fish. The effect of nickel on the biochemical and haematological parameters of fish have also been studied. Agrawal *et al.* (1979) reported changes in haematological parameters of *Colisa fasciatus* after nickel intoxication. Chaudhry (1984) has studied the changes in glycogen content of liver and muscle after exposure to nickel. The effect

of industrial effluent containing nickel and other heavy metals on the energy reserves of fish is described by Shafi (1980). Muramoto (1983) has studied the toxicity of nickel chloride and nickel sulphate to carp, *Cyprinus carpio*, in the presence of complexans. Detailed information on the toxicities of heavy metals in general are available in the work of Atchison *et al.* (1987) and Matheis (1988). Other workers such as Sunderman *et al.* (1989), Milicevic & Milicevic (1989), Cartana *et al.* (1992) and Snow (1992) have studied the effect of different forms of nickel on rats. Adams *et al.* (1992) have reported toxic effects of nickel chloride and nickel sulphate on daphnia.

In this investigation tilapia, *Oreochromis niloticus*, was used as a test animal because it is an important fish species in both natural and man-made aquatic environments in Saudi Arabia, and the culture of this species has substantially increased during recent years. The author has made an attempt to evaluate the toxicity of nickel chloride by recording mortality and changes in behaviour and blood parameters after exposure to nickel. Haematological parameters have proven especially valuable in monitoring the response of fish to sublethal concentrations of toxicants (McLeay & Gordon, 1977, Srivastava & Agrawal, 1979).

MATERIALS AND METHODS

Healthy specimens of *Oreochromis niloticus* were procured from a fish farm located at Deerab about 60 km south-west of Riyadh. The average length and weight of fish was 13.78 cm and 41.2 g, respectively. 150 fish were kept in a glass aquarium of 480 l capacity and left for 4 weeks to acclimatize to laboratory conditions. During the period of acclimation the fish were fed commercial fish food to satiety twice daily. The fish were unfed during the period of exposure to nickel chloride. Records of the temperature, pH and dissolved oxygen level of the water were kept.

When the acclimation period was over ten fishes, randomly selected from stock, were transferred to an aquarium containing 30 l water in which nickel chloride was dissolved. For testing lethal concentrations tanks containing 26, 26.5, 27, 27.5, 28 and 28.5 mg/l were prepared by adding a known volume of stock solution of $\text{NiCl}_2 \cdot 6\text{H}_2\text{O}$ to each tank. A control series was run with the same number of fish and the same volume of water but without nickel. All aquaria were aerated with mechanical pumps. The medium of all tanks was completely renewed after every 24 hours. The fish were exposed for a total of 96 hours and the mortality for each concentration was registered separately. LC-50 for 96 hours was computed by probit analysis, according to the method of Finney (1971). Changes in behaviour of the fish during the period of exposure to lethal concentrations were recorded.

In another set of experiments, five fish were exposed to each of three sublethal concentrations (1.5, 3.0 and 5.0 mg/l) for 4 days. A control set having the same number of fish and the same volume of water (15 l) was run simultaneously for comparison. Changes in behavioural patterns of the fish were observed and recorded following the methods of Henery & Atchison, (1986). The observations were made for 30 minutes for all five fishes in each tank at 24 hr, 48 hr, 72 hr and 96 hr of exposure. Behavioural observations of both control and treated groups were made at 8 am–10 am on day 1, 10 am–12 noon on day 2, 1200–2 pm on day 3 and 2 pm–4 pm on day 4. Behavioural observations were made at different times so that diurnal

Table 1. Behaviour of *Oreochromis niloticus* monitored in the present study (after Henery & Atchison, 1986).

| Behaviour | Description |
|----------------|--|
| Cough | Rapid, repeated opening and closing of mouth and opercular covering with partial extension of fins |
| Yawn | Wide opening of mouth and hyperextension of fins |
| S-jerk | Movement of body sequentially from head to tail |
| Partial-jerk | Movement of head or tail only |
| Fin-Flickering | Repeated extension and contraction of dorsal fin |
| Burst Swimming | Sudden and rapid movements (Forward) |
| Chafe | Rubbing of the body against the inanimate object |
| Nudge | Movement of the fish towards another fish |
| Nip | Bite |

fluctuations in activity would not be confounded with toxicant effect. The sequence of tanks in which the observations were made was also randomized.

At the end of exposure, all the fishes from each concentration were sacrificed and their blood was collected in heparinized vials by cutting the caudal peduncle. Samples which clotted were discarded. For differential leucocyte count, smears were immediately prepared, air dried, fixed in methanol for five minutes and stained with Leishman or Giemsa. Leucocytes were identified by morphological and staining characteristics. Smears were examined at $\times 1000$. A minimum of five smears and 500 cells were counted for each concentration. Erythrocyte and leucocyte counts were made with Neubaur haemocytometer using standard diluents. Haemoglobin was estimated by the method of Blaxhall & Daisley (1973). The blood samples were centrifuged at 6000 rpm for 10 minutes in haematocrit tubes for the estimation of haematocrit value. The erythrocytic indices such as mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were calculated according to the methods of Ghai (1986). Five samples of blood collected separately were analysed for each parameter from control and treated groups. The data were subjected to statistical analysis (Student's 't' test) to test the differences between the experimental and control means.

RESULTS

The 96 hours LC-50 computed from the Fig. 1, drawn from the probits of kill against Log_{10} concentrations of nickel chloride was 27.2 mg/l. Fish exposed to lethal concentrations (26.0, 26.5, 27.0, 27.5, 28.0 and 28.5 mg/l) of nickel chloride initially showed hyperexcitation, restlessness, abnormal swimming, secretion of mucus, followed by loss of balance and finally death.

Substantial changes in behaviour of fish exposed to sublethal concentrations (1.5, 3.0, 5.0 mg/l) of nickel were recorded. The most significant effect was on respiration ($P < 0.01$) manifested as an increase in the frequency of coughs and yawns. The

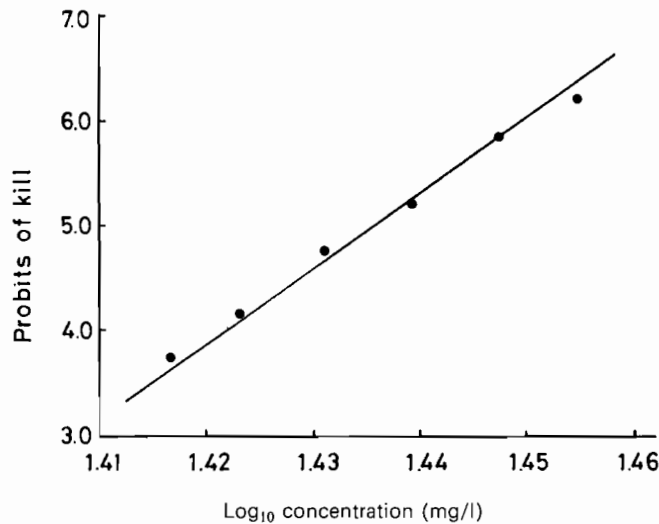


Fig. 1. Relationship between probits of kill and logarithm of nickel concentrations (mg/l).

increase was greater in the initial 24 hours of exposure but remained higher than the control level throughout the period of investigation (Fig. 2).

Aggressive interactions like nudging and nipping were also increased in nickel-exposed fish (Table 2). Frequencies were higher at higher concentrations and in first 24 hours of exposure and remained higher than the control throughout the period of exposure (Fig. 2). Toxicant exposed fish secreted mucus in large quantities. Discomfort movements (fin flickering, partial jerk, S-jerk and burst swimming) were observed in nickel exposed fish (Table 2 and Fig. 2). Increase in the frequency of fin flickering was insignificant ($P > 0.01$) at low concentrations but significant at higher level of intoxication. Frequency of partial jerk was increased significantly at all levels

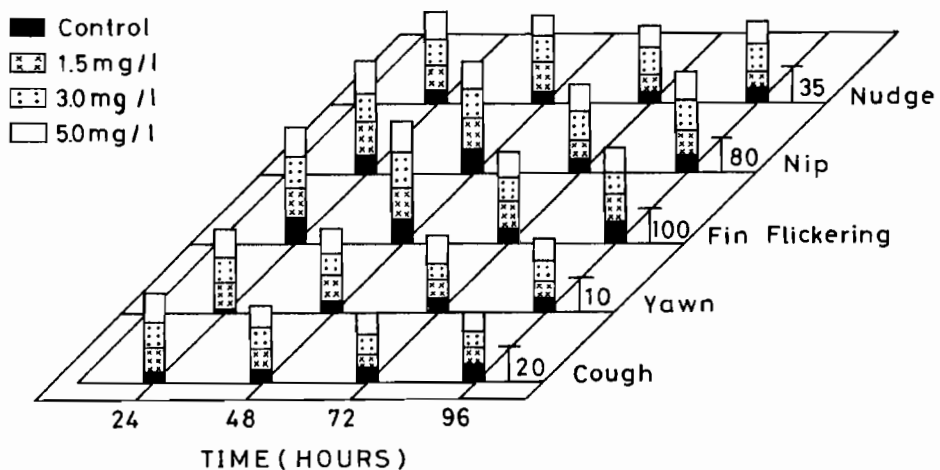


Fig. 2. Frequency of occurrence of some behaviour of *Oreochromis niloticus* at different time intervals after exposure to sublethal concentrations of nickel.

Table 2. Frequency of occurrence of different behaviour after nickel intoxication. Data are summations of frequencies of five fishes per tank for four daily 30 minute observation periods (total 2 hours) averaged for two replications (\pm values are standard errors).

| Parameters | Concentrations | | | |
|----------------|-----------------------|--------------------|--------------------|--------------------|
| | 0.0 mg/l (Control) | mg/l 1.5 | mg/l 3.0 | mg/l 5.0 |
| Cough | 34.5 \pm 1.499 | 43.5* \pm 0.502 | 46.0 \pm 0.997 | 51.0* \pm 0.997 |
| Yawn | 14.0 \pm 0.997 | 23.5* \pm 0.499 | 22.5 \pm 0.499 | 27.0* \pm 0.502 |
| Fin-flickering | 253.5 \pm 4.999 | 281.5 \pm 5.501 | 322.5* \pm 5.501 | 304.5* \pm 2.496 |
| Partial-Jerk | 42.0 \pm 0.499 | 53.5* \pm 0.499 | 66.5* \pm 2.496 | 65.0* \pm 2.998 |
| S-Jerk | 27.0 \pm 0.997 | 32.0 \pm 0.997 | 38.5 \pm 4.479 | 44.0 \pm 7.992 |
| Burst Swimming | 3.5 \pm 0.499 | 8.5* \pm 1.499 | 8.0* \pm 1.994 | 7.0* \pm 0.997 |
| Chafe | 47.5 \pm 2.496 | 82.5* \pm 4.497 | 72.5* \pm 1.499 | 71.0* \pm 1.994 |
| Nudge | 52.0 \pm 0.997 | 86.5* \pm 0.499 | 107.5* \pm 1.499 | 84.0* \pm 0.994 |
| Nip | 175.0 \pm 7.997 | 245.5* \pm 3.499 | 263.5* \pm 1.499 | 257.0* \pm 0.499 |

* Significant difference between the experimental and control means ($P < 0.01$).

of exposure. Significant increase in the frequency of S-jerk was registered only at higher concentrations (Table 2).

The haematological observations presented in Table 3 indicate that the red cell count, haemoglobin concentration and haematocrit values were increased in the experimental group. The leucocyte count, on the other hand, was decreased. Values of the erythrocytic indices such as MCV, MCH and MCHC were changed but the changes were insignificant ($P > 0.01$). Data pertaining to differential leucocyte counts presented in Table 4 indicate that the percentages of thrombocytes and lymphocytes decreased after nickel treatment. There was an increase in the percentage of monocytes, neutrophils and eosinophils. These changes in the percentage of different cells appeared to be concentration dependant.

DISCUSSION

Nickel is toxic to the tilapia, *Oreochromis niloticus*, and causes death at certain concentrations. However, nickel appears to be less toxic to this species than other heavy metals like cadmium and chromium. The median lethal concentration (Lc-50)

Table 3. Changes in haematological parameters of fish exposed to sublethal concentrations of nickel chloride. Data are mean of five replicates and \pm values are standard errors.

| Parameters | Concentrations | | | |
|----------------------------|-----------------------|--------------------|--------------------|--------------------|
| | 0.0 mg/l (Control) | mg/l 1.5 | mg/l 3.0 | mg/l 5.0 |
| RBC ($10^6/\text{mm}^3$) | 1.314 \pm 0.027 | 1.480 \pm 0.031 | 1.468 \pm 0.014 | 1.514* \pm 0.228 |
| WBC ($10^3/\text{mm}^3$) | 38.48 \pm 0.590 | 37.44 \pm 0.631 | 36.22 \pm 0.818 | 35.02 \pm 0.527 |
| Haemoglobin (g/100 ml) | 6.54 \pm 0.161 | 7.72 \pm 0.072 | 7.86* \pm 0.174 | 8.0* \pm 0.103 |
| Haematocrit (%) | 32.32 \pm 0.286 | 35.80 \pm 0.586 | 36.66* \pm 0.832 | 36.98* \pm 0.127 |
| MCV (fl) | 246.48 \pm 6.574 | 241.72 \pm 6.816 | 249.62 \pm 3.376 | 244.69 \pm 8.202 |
| MCH (pg) | 49.82 \pm 1.290 | 52.10 \pm 1.038 | 53.60 \pm 1.724 | 51.96 \pm 0.930 |
| MCHC (%) | 20.23 \pm 0.492 | 21.59 \pm 0.425 | 21.51 \pm 0.872 | 21.68 \pm 0.604 |

* Significant difference between the means of control and treated fish ($P < 0.01$).

Table 4. Differential leucocyte count of fish exposed to nickel chloride. Values are means of five observations. \pm values indicate the standard errors.

| Cell type (%) | Control | 1.5 mg/l | 3.0 mg/l | 5.0 mg/l |
|---------------|-----------------|------------------|------------------|------------------|
| Thrombocytes | 40.8 \pm 1.49 | 37.2* \pm 1.65 | 35.8* \pm 1.49 | 36.0* \pm 1.38 |
| Lymphocytes | 50.0 \pm 1.64 | 44.2* \pm 1.68 | 42.4* \pm 1.72 | 42.8* \pm 1.88 |
| Neutrophil | 3.2 \pm 0.37 | 9.6* \pm 0.92 | 11.2* \pm 1.16 | 12.0* \pm 1.14 |
| Eosinophil | 1.0 \pm 0.31 | 1.8 \pm 0.66 | 3.2* \pm 0.58 | 2.2** \pm 0.86 |
| Basophil | 1.0 \pm 0.31 | 1.6** \pm 0.25 | 1.8** \pm 0.37 | 1.8 \pm 0.86 |
| Monocyte | 2.6 \pm 0.25 | 3.6* \pm 0.25 | 3.6** \pm 0.51 | 3.4 \pm 0.81 |
| Unidentified | 1.6 \pm 0.25 | 1.6 \pm 0.25 | 2.0 \pm 0.44 | 1.8 \pm 0.37 |

* Significant difference to control ($P < 0.001$).

** Significant difference to control ($P < 0.01$).

registered for nickel chloride (27.2 mg/l) was much higher than for cadmium (5.2 mg/L), and slightly higher than for chromium (23.6 mg/l) (Al-Akel *et al.* 1988 and Al-Kahem—in press). The median lethal concentration (Lc-50) for nickel for different species of fish vary from 7.0 to 118.3 mg/l (Taylor *et al.* 1985, Atchison *et al.* 1987 and Matheis, 1988) and the value obtained in the present study is within this range.

The fish exposed to lethal concentrations showed abnormal swimming behaviour, convulsions, jerking movements, hyperventilation and increased secretion of mucus. These behavioural changes were more prominent at higher concentrations (28.0 and 28.5 mg/l) than lower concentrations (26.0 and 26.5 mg/l). Ultimately fish exhibited loss of balance, swam upside down and finally succumbed. This hyper-excitation of the fish may be because of physiological disturbances caused by the nickel. Increased secretion of mucus is a typical response to the irritating effect of toxicants; coating the body in order to reduce body contact with a stressful environment. The fast and abrupt movements may represent an attempt to escape from the toxic medium. Loss of balance may be caused by effects of the toxicant on the lateral line system. Because of the location of the swimbladder fish are inherently unstable and will turn upside down with loss of motor control and with the approach of death.

Changes in the behavioural pattern of fish exposed to sublethal concentrations of nickel were also registered. The most pronounced and significant effect was on respiration ($P < 0.01$). Raised swimming activity stimulated by the toxicant would increase the requirement for oxygen, to meet the energy demand of elevated muscular activity. Furthermore deposition of mucus on the gills would reduce the gaseous exchange through them, thus reducing the oxygen supply. Consequently the fish will cough and yawn more to clear the gills of mucus to enhance the exchange of gas. It is reported that yawn and cough have a clearing effect on gills (Henery & Atchison, 1986; Al-Kahem *et al.*, 1990). Steady reduction in the frequencies of cough, yawns and other behaviour (Fig. 2) after prolonged exposure to nickel may be attributed to increased resistance and adaptation of fish to the stress caused by the toxicant.

Changes in discomfort movements like fin flickering, jerking and burst swimming, and aggressive behaviour such as nip and nudge may be a response to the irritating effect of the toxicant and disturbances in physiological mechanisms which according to Marler & Hamilton (1966) initiate, maintain and terminate such behaviour. Fish exposed to nickel chloride exhibit increased muscular exertion possibly leading to hypoxia. This could result in compensatory erythropoiesis with consequent

production of polycythemia and concomitant increase in haemoglobin and haematocrit values. That fish produce new erythrocytes and synthesize haemoglobin under hypoxic condition is reported by Agrawal *et al.* (1979). Polycythemia is also seen in rats after the exposure to nickel. (Jasmin 1973; Jasmin & Solymass 1975; Jasmin & Riopelle 1976; Hopfer & Sunderman 1978; Hopfer *et al.* 1978).

Reduction in the total WBC count after exposure to nickel is attributable to a reduction in numbers of circulating thrombocytes and lymphocytes (Table 4). The mechanism inducing such a lymphopenia might be a diminution in delivery of lymphocytes to the circulatory system through a reduced lymphocyte production, or alternatively a rapid destruction of cells and/or increased rate of peripheral removal of lymphocytes. The most probable explanation is that there is a failure of lymphocyte production together with the dissolution of cells. Many workers (Mustafa & Murad 1984; Ball & Slicher 1962; Ellis 1977; Murad & Houston 1988) attributed such lymphopenias to the lysis of lymphocytes after exposure to toxicants. Leucopenia was observed in the fish, *Colisa ilisa* by Agrawal *et al.* (1979) after exposure to nickel. Some other investigators such as Agrawal & Srivastava (1976), Mishra & Srivastava (1979) and Srivastava & Agrawal (1979) have registered leucopenia in fish after exposure to other metal ions toxicants and attributed it to the lysis of lymphocytes.

The thrombocytopenic response in *Oreochromis niloticus* was less profound than the lymphopenic one after exposure to nickel chloride. A similar response was recorded by Mustafa & Murad (1984) in *H. fossilis* following exposure to DDT, and reduction in the number of thrombocytes in gold fish (*Carassius auratus*) was reported by Murad & Houston (1988) after exposure to cadmium.

Ellis (1981) suggested that in fishes lymphopenia is commonly accompanied by neutrophilia and this was seen in the present study. Neutrophilia in gold fish was recorded by Murad & Houston (1988) after cadmium intoxication. Teleostean neutrophils are formed in a granulopoietic area of the kidney (Ellis 1977) and are phagocytic in nature. Neutrophilia recorded in fish exposed to metal ions may be attributed to increased longevity in circulation, release from storage sites, or to differential sensitivity of lympho- and granulopoietic cells. Similar considerations may also apply to the eosinophilia and basophilia encountered following exposure to nickel.

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تأثير النيكل على مكونات دم وسلوك سمك البلطي *Oreochromis niloticus*

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خلاصة

لقد تم غمر عدة عينات من سمك البلطي في تراكيز متعددة من كلوريد النيكل تتراوح بين ٢٦-٢٨,٥ ملجرام/لتر. وتبين من هذه الدراسة أن التركيز الحرج لهذا العنصر قد بلغ ٢,٢ ٢٧ ملجرام/لتر. وتبين كذلك أن درجة تأثير الأسماك بالتراكيز المختلفة تباينت من الاضطرابات النفسية والسلوك العدواني بين أفراد الأسماك في الحوض إلى نقص في عدد الكريات الدموية البيضاء وزيادة عدد كرات الدم الحمراء، كما سُجل إرتفاع في تركيز الهيموجلوبين والهيماتوكريت. وسجل العد التفاضلي للكريات البيضاء انخفاضاً في عدد الصفائح الدموية والكريات اللبناوية وزيادة في الكريات المحببة.