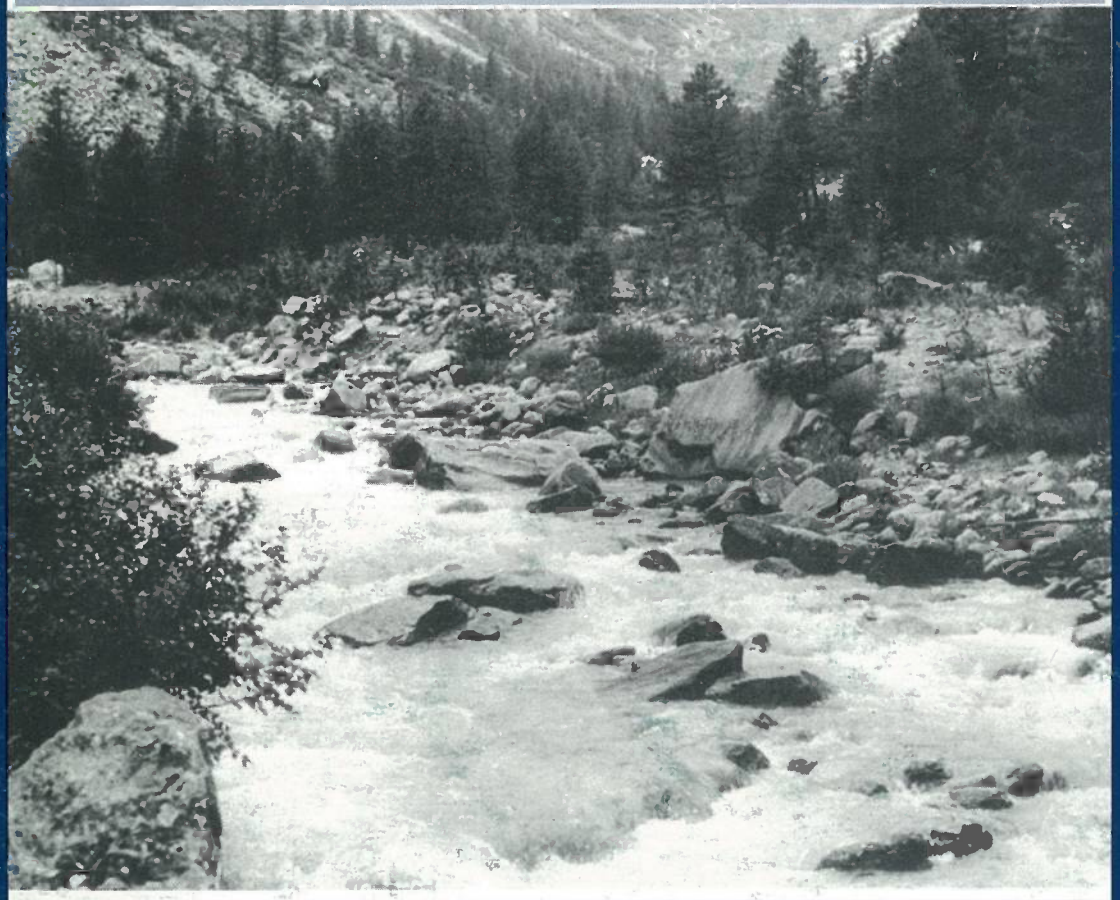
Water quality criteria for European freshwater fish

Report on nickel and freshwater fish



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FOOD AND AGRICULTURE ORGANIZATION OF THE UNITED NATIONS

Water quality criteria for European freshwater fish

Report on nickel and freshwater fish

Prepared by the
EIFAC Working Party on Water Quality Criteria
for European Freshwater Fish

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PREPARATION OF THIS DOCUMENT

This is the fourteenth review of the EIFAC Working Party on Water Quality Criteria for European Freshwater Fish.

For the preparation of this report, the following experts were appointed to the Working Party:

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The Working Party used the same general basis for their work as that on which they had agreed for the preparation of their first report that:

"Water quality criteria for freshwater fish should ideally permit all stages in the life cycles to be successfully completed and, in addition, should not produce conditions in a river water which would either taint the flesh of the fish or cause them to avoid a stretch of river where they would otherwise be present, or give rise to accumulation of deleterious substances in fish to such a degree that they are potentially harmful when consumed. Indirect factors like those affecting fish-food organisms must also be considered should they prove to be important".

This report was prepared by D. Calamari and J.F. de L.G. Solbé, and the conclusions and tentative water quality criteria were approved by the Thirteenth Session of EIFAC (Aarhus, 23-30 May 1984). The assistance of Dr. G. Mance (UK) who commented on the final draft is gratefully acknowledged.

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ABSTRACT

This report, prepared by the European Inland Fisheries Advisory Commission Working Party on Water-Quality Criteria for European Freshwater Fish, critically reviews the literature on the occurrence and effects of nickel in fresh water. It lists and discusses the sources of nickel, and its chemistry and analysis in fresh water. The mode of action of nickel and the factors which affect its short- and long-term toxicity to the various life-cycle stages of fish are dealt with in detail. Similar information for plants and invertebrates is considered and evidence for the accumulation of the metal in animals and plants is reviewed.

The tentative water-quality criteria proposed do not distinguish between salmonid and non-salmonid waters but do recommend separate criteria for hard and soft waters. The conditions under which more or less stringent standards may be appropriate are indicated. To protect fresh waters, the average aqueous concentration of "soluble" nickel should not exceed 0.01 mg Ni/l and the 95 percentile should not exceed 0.03 mg Ni/l in soft water (20 mg/l as $CaCO_3$). In hard water (320 mg/l as $CaCO_3$), it is proposed that the corresponding concentrations of nickel should be 0.04 and 0.12 mg/l respectively. These concentrations are considered to be satisfactory also for the protection of the majority of other freshwater organisms. Higher concentrations might also be satisfactory where organic complexing agents are present.

CONTENTS

1. INTRODUCTION

1.1 Sources and Uses of Nickel

Nickel (Ni) is a metallic element with an atomic weight of 58.71. The average concentration in the earth's crust is estimated to be 60-90 mg/kg but it is relatively depleted in acidic rocks and enriched in basic and ultrabasic rocks. Nickel has a similar ionic radius and electronegativity to iron, cobalt and magnesium and can therefore substitute for these elements in various minerals. The sulphides ores are particularly important commercially, the principal European producers being Norway, Finland, the German Democratic Republic, Albania and Greece. Further details of the geochemistry and chemistry of nickel have been published by Nriagu (1980) and National Research Council of Canada (1981).

Nickel is used in electro-plating, the production of alloys, and of alkaline storage batteries. It is released into the environment during these manufacturing processes, and particularly during mining and smelting and in the combustion of fossil fuels (crude oil can contain tens of milligrams of nickel per kilogram). Nickel can also occur in the run-off from roads.

1.2 Chemistry of Nickel in Fresh Water

Nickel is the last metallic element in the first transition series of the periodic chart of the elements. Several oxidation states of the metal are known, however the 0 and 2+ oxidation state are the most common (Cotton and Wilkinson, 1972). The predominant oxidation state in natural waters in Ni^{2+}.

A review on nickel speciation in soil/water system has been made by Richter and Theis (1980).

Complexes of naturally occurring ligands are formed to a small degree according to the stability constants, with the following decreasing scale: $OH^- > SO_4^{2-} > Cl^-$, and the free aquo species dominates at the neutral pH range found in most aerobic waters (Morel, McDuff and Morgan, 1973).

Baes and Mesmer (1976) studied the distribution of nickel species as a function of pH and, on the bases of the values for the hydrolysis reactions of the different aquo species, stated that in waters of pH 5 to 9 the free ion Ni^{2+} is almost the only form present.

The solubility in water of most of the natural salts of nickel, including carbonate, is high (>1 g/l) , therefore the metal does not give rise to precipitates under normal conditions. On the other hand the solubility of nickel sulphide is quite low (<5 mg/l) and in anaerobic conditions sulphide is the major factor controlling the solubility of the metal (Sillen and Martell, 1971).

Nickel forms stable complexes with many organic ligands, such as amino-acids, EDTA, NTA, etc; the stability constants are reported by Smith and Mansell (1975). Humic and fulvic acids also form complexes (stability constants in Mantoura, Dickson and Riley, 1978) and their capacity to absorb nickel is proportional to the concentration of dissolved nickel ions; according to Chowdhury and Bose (1971) the ratio of metal ions, in humus to those in water is about 950.

The ferrous metals, due to their high electronegativity, tend to be strongly adsorbed onto clay minerals. Nickel is easily adsorbed on clay minerals also and concentrated in the finest fraction (Allan and Crook, 1972). Due to that, property sediments could be regarded as a possible reservoir of nickel, as for many other metals. Morris (1975) reported that metals tend to dissolve out of the sediments and into the water under field conditions at low pH and high Eh.

Nickel can therefore occur in the aquatic environment as its soluble salts, adsorbed on suspended solids and in the form of organic complexes. The metal adsorbed onto clays and other kinds of suspended solids cannot, *a priori* be readily available for toxicological processes; the fraction of nickel which forms organic complexes is difficult to quantify which makes it impossible to evaluate its contribution to toxicological processes.

Taking into account all these considerations, water-quality criteria for nickel should therefore be expressed as total soluble nickel (defined, for convenience as those forms able to pass through a 0.45 um pore-size filter).

Unless otherwise stated the data given in this report refer to total soluble nickel.

1.3 Analytical Methods

The main analytical techniques employed routinely for determining the concentration of nichel in natural and polluted waters are atomic absorption spectroscopy (AAS) using flame excitation (Christian and Feldman,1971;Burrell, 1974), preceded if necessary by solvent extraction and concentration (Kinrade and Van Loon, 1974; Stoeppler, May and Mohl, 1978; Danielsson, 1980), and flameless AAS graphite furnace (Manning and Ediger, 1976; Danielsson *et al.*, 1980). Colorimetric techniques are also available (APHA, 1981).

For atomic absorption spectroscopy the detection limits vary, according to different authors and techniques, in a range from 0.010 to 0.0002 mg/l, while colorimetric methods can give a detection limit as low as 0.001 mg/l.

Other techniques have been used including neutron activation, x-ray methods, atomic fluorescence, mass spectometry and electrochemical methods (pulse polarography, stripping voltammetry) (Stoeppler, 1980).

Several analytical methods for the determination of nickel in biological samples are described in the literature and Stoeppler (1980) recently prepared an extensive review, which included analytical determinations in water. Among the most utilized techniques are those which involve acid dissolution of the samples followed by digestion combining heat and pressure (Julshamn and Braekkan, 1975; Stoeppler, 1980). Similar types of wet digestion are also used for the determination of nickel in sediments.

1.4 Occurrence in Water and Sediments

An extensive review on distribution and behaviour of nickel in the aquatic environment has been recently made by Snodgrass (1980) who mainly drew on North American data. Kopp and Kroner (1967) reported dissolved nickel in 16% of 1 577 water samples from various regions of the USA and gave a mean concentration of 0.019 mg/l. Nickel in uncontaminated waters was considered to vary from 0.003 to 0.017 mg/l. In another survey made in 1962 (NAS, 1975) on tap water the concentrations ranged from <0.001 to 0.059 mg/l with a mean of 0.005 mg/l. These data have been confirmed by McCabe (1974) who reported an average nickel concentration of 0.0048 mg/l.

Among the measurements of nickel in European waters, a report of the Italian Water Research Institute (IRSA, 1980) gave values under the detection limit of flameless atomic absorption spectrophotometry (0.002 mg/l) for about seventy Italian lakes. Gaggino (1982) reported, for unfiltered water, a mean concentration of 0.003, 0.0027 and 0.0032 mg/l, in three sampling stations located all along the river Po in northern Italy, while water filtered through membranes of 0.45 um pore-size contained nickel at concentrations below the detection limit of flameless AAS.

In 31 rivers in the UK the range of mean concentrations of dissolved nickel was from 0.012 to 0.073 mg/l for relatively polluted waters (yet which may still permit the passage of migratory fish and the existence of put-and-take fisheries) and from 0.0007 to 0.0037 mg/l for clean rivers (Mance and Yates, 1984).

Mean concentrations of the dissolved metal in other European countries were as follows: Neckar - 0.0308 mg/l (Lodemann and Bukenberger, 1973); Rhine 0.0144 mg/l (Brinkmann, 1973) and 0.013 mg/l (Bouquiaux, 1974); Weser 0.0162 and Scheldt 0.016 mg/l (Bouquiaux, 1974); Vesdre 0.0151 mg/l (Boelen and de Boeck, 1976).

Other data on the concentrations of nickel in the sediments of European rivers are available. Laskowski *et al.* (1976) found values above 0.1 mg/kg in rivers from the Rhine-Main area, a densely populated and industrialized region. Solomons and Mook (1977) reported lower values, from 0.019 to 0.059 mg/kg, in Dutch estuarine sediments. These last values are slightly in excess of those considered as the normal range, for example by Fitchko and Hutchinson (1975).

2. LETHAL EFFECTS ON FISH

2.1 Mode of Action

No precise descriptions of the biochemical basis of nickel toxicity have been reported in the literature. However metabolic studies in animals indicated that nickel, in the form of the divalent ion, was bound to a variety of molecular structures such as nucleic acids and proteins.

Inhibition of ATP-ase activity by nickel has been reported both *in vivo* and *in vitro* on enzymes from various sources; effects on several other enzymes were also reported (see the review of Mushak, 1980).

Very few data exist on fish; Hughes, Perry and Brown (1979) found damage to the structural components of the gill secondary lamellae of rainbow trout (*Salmo gairdneri*) with a consequent impairment of the ability of fish to transfer oxygen from the water to their blood, after short-term treatment with 0.05 of the 48-h LC50. Arillo *et al.* (1982) measured several biochemical parameters in rainbow trout after six months exposure to 0.02 of the 48-h LC50. Total liver glucides, total liver proteolytic activity, total liver proteins and sialic acid in the gill are known to change when several physiological disturbances are taking place, for example when homeostasis is maintained at heavy energetic expense. These changes have therefore been considered as indicators of stress. Moreover these determinands are also known to be affected by a specific metal action. After a six-month treatment a statistically significant decrease of sialic acid in gill tissue was found, but the levels recovered to normal after three months in clean water.

The depletion of sialic acid may negatively affect osmoregulation and immunological properties; mucus is in fact known to contain immuno-globulins and some molecules characterized by lysozyme-activity. Fish exposed to nickel showed a marked drop in glucidic stores, particularly in mature males, that returned to normal after a three-month period of recovery. This finding is in agreement with nickel-induced hyperglycaemia in mammals (Sunderman, 1977).

Since other -SH inhibitors caused analogous metabolic alterations to the liver glucidic contents the observed effect may be caused by direct metal interaction on both the cell membranes and the thiolic fraction of enzymes in the pancreas. Proteolytic activity and total glucides levels are regulated by hormones and an inversely proportional relationship among the two determinands was found in the control animals while treated male fish showed statistically different results. The change in this relationship may be due to lysosomal proteases, which being metallo-enzymes could be inhibited by high concentrations of metal (Waters *et al.*, 1975).

2.2 Factors Affecting Acutely Lethal Levels

2.2.1 Introduction

A number of factors can influence the toxicity of metals to fish, but only a few of them have been studied with nickel. Much of the data referred to in the following section is based on studies with non-European species of fish, although a few of the species are now present and important in European waters (e.g., rainbow trout, *Salmo gairdneri*).

2.2.2 Hardness

Pickering and Henderson (1966) compared the toxicities of nickel chloride in waters of two hardness characteristics (total hardness 20 mg/l and 300 mg/l as $CaCO_3$). The 96-h LC50 was 4.9 mg/l for fathead minnow (*Pimephales promelas*) and 5.3 mg/l for bluegill sunfish (*Lepomis macrochirus*) in soft water and 43.5 mg/l and 39.6 respectively in hard water. Rainbow trout showed an increase of four-fold in sensitivity from hard to soft water, the 48-h LC50 changing from about 80 to about 20 mg/l (Brown, 1968). Recently Calamari (personal communication) confirmed these data; he compared the toxicity of nickel at a single pH (7.4) to rainbow trout (100 mm in length) in hard and in soft water (total hardness 320 and 20 mg/l as $CaCO_3$ respectively). At 15°C the 48-h LC50s were 53.6 mg Ni/l in the hard water and in soft water 17.2 mg/l. Birge and Black (1980) found that in water of hardness 100, 125 and 174 mg/l as $CaCO_3$ the LC50s to rainbow trout exposed from fertilization to 4 days post-hatch were 0.05, 0.06 and 0.09 mg/l respectively.

2.2.3 Temperature

Rehwoldt, Bida and Nerrie (1971) and Rehwoldt *et al.* (1972) examined the effects of temperature on the toxicity of nickel to six warm-water species of fish: banded killfish (*Fundulus diaphanus*), striped bass (*Roccus saxatilis*), pumpkin seed (*Lepomis gibbosus*), white perch (*Roccus americanus*), American eel (*Anguilla rostrata*) and carp (*Cyprinus carpio*). There was a wide range of sensitivity among these species, with 96-h LC50 values at 17°C ranging from 6.2 to 46.2 mg Ni/l, while at 28°C each species showed very little variation from its sensitivity at the lower temperature.

2.2.4 Species

Among the six species tested by Rehwoldt *et al.* (1972) at 28°C and at a hardness of 55 mg/l as $CaCO_3$ *Roccus saxatilis* and *Lepomis gibbosus* were the most sensitive, having 96-h LC50s of 6.3 and 8.0 mg Ni/l respectively while *Fundulus diaphanus* was the most tolerant (46.1 mg Ni/l). *Roccus americanus*, *Anguilla rostrata* and *Cyprinus carpio* were intermediate in their response but relatively sensitive, the 96-h LC50s being 13.7, 13.0 and 10.4 mg Ni/l respectively.

In static tests, in which the concentration of nickel was not measured, *Pemephales promelas*, *Lepomis macrochirus*, *Carassius auratus* and *Lebistes reticulatus* were demonstrated by Pickering and Henderson (1966) to be of a similar level of sensitivity to this last group of fish, with 96-h LC50s, in a softer water (20 mg $CaCO_3$/l) of 4.9, 5.3, 9.8 and 4.5 mg Ni/l, respectively. Rainbow trout, however, are apparently less sensitive, with a 48-h LC50 in flow-through tests in soft water of about 20 mg/l (Brown, 1968 and Calamari, personal communication).

These last data are in agreement with field experiments conducted by Hale (1977), who found a 96-h LC50 to rainbow trout of 35.5 mg Ni/l in continuous-flow tests, using water with an alkalinity of 82-132 mg/l as $CaCO_3$.

2.2.5 Age and size

Data from the UK Water Pollution Research Laboratory (Great Britain, Ministry of Technology, 1967) demonstrated that for alevins of rainbow trout 14 days old the 12-d LC50 was 5 mg/l in hard water, and in a similar experiment, Great Britain, Ministry of Technology (1969), confirmed the high sensitivity of young stages, the 20-d LC50 being 1.3 mg Ni/l, and no further mortalities occurred within the 56-d exposure period.

2.2.6 Joint action of nickel and other poisons

Brown and Dalton (1970) measured the 48-h LC50 to rainbow trout of copper, zinc and nickel, and mixtures of the three, in a hard water (240 mg/l as $CaCO_3$) in the ratio of 1:1:1 of the respective proportions of their 48-h LC50 values and found that the joint effect of the three was approximately additive. Marking (1977) also found that the joint action of such a mixture was approximately additive (unspecified LC50). Weinstein and Anderson (1978), using zebra fish (*Brachydanio rerio*), showed that copper and nickel were more than additive at lethal levels, and also reported (Anderson, Horovitch and Weinstein, 1979) that this was markedly affected by the relative proportion of each in the mixture - the higher the proportion of nickel, the lower the percentage mortality. However, Muska and Weber (1977) reported briefly that, in 7-d tests on the effect of copper and nickel and their mixture on the growth and food consumption of juvenile guppy at 7°C and 25°C, interaction between copper and nickel was slightly more than additive with a restricted ration.

Data provided by F.S.H. Abram (personal communication) for the survival of rainbow trout in mixtures of nickel and chromium in a hard water (hardness 270 mg/l as $CaCO_3$) at 15°C show that, for the 96-h LC50 in the ratio of 3:1 of the proportions of their LC50 values, chromium and nickel exhibit additive joint action.

Anderson, Horovitch and Weinstein (1979) found that the lethal effects of mixture of nickel and vanadium became progressively less than additive as the proportion of vanadium increased. In contrast, Anderson, Horovitch and Weinstein (1979) found that when the concentrations of nickel and vanadium increased in proportion to that of phenol in tertiary mixtures, the lethal effect of the mixture became progressively (2-fold) more than additive.

From these data it can be concluded that the acute lethal toxicity of mixtures of nickel and other poisons is between 0.6 and 2 times that predicted from the sum of the proportions of the respective toxic units of the constituents, but that the majority of the cases lie near to 1, i.e., simple additive joint action occurs.

From the limited information available it appears that hardness is the major factor which markedly affects the toxicity of nickel to fish, while variations in temperature apparently do not influence toxicity. Interspecific variations of sensitivity between fish are within a range of less than one order of magnitude under comparable physico-chemical test conditions.

2.3 Developmental Stages and Life-Cycle Studies

Shaw and Brown (1971) observed no effect on rainbow trout eggs fertilized in 1 mg Ni/l and then maintained in clean water. Pickering (1974) in a life-cycle study on fathead minnow found that nickel concentrations of 0.38 mg Ni/l and lower (0.18 and 0.08) caused no adverse effect on survival, growth and reproduction. However a nickel concentration of 0.73 mg/l had a statistically significant effect on the number of eggs produced per spawning and on the hatchability of these eggs, although it did not affect the survival and growth of the first generation of fish. The dilution water had a hardness of 210 mg $CaCO_3$/l, pH 7.8 and an average temperature 18°C.

For carp (*Cyprinus carpio*) eggs and larvae (Blaylock and Frank, 1979) the 72-h LC50s were 6.1 and 8.4 mg/l respectively while the 257-h LC50 for larvae was 0.75 mg Ni/l at a water hardness of 128 mg $CaCO_3$/l, a pH of 7.4 and a temperature of 25°C. In the same experiment a concentration of 3 mg Ni/l caused an increased incidence of abnormal larvae (23% compared with 8.6% in the controls); also, 32.7% of embryos failed to hatch, compared with 5.9% in the controls.

Birge *et al.* (1978) exposed rainbow trout and largemouth bass (*Micropterus salmoides*) to 11 trace metals found in coal, from fertilization to four days after hatching. At 12-13°C this gave a period of exposure of 28 days for rainbow trout and 8 days for bass. The LC50 values for these periods were 0.05 mg Ni/l for rainbow trout and 2.06 mg/l for bass, but concentrations perhaps as much as 100 times lower than these were shown to kill small percentages of the 100 test organisms used at each concentration. Calculations of the LC1 values for nickel were 0.0006 mg/l and 0.0097 mg/l for the trout and bass respectively, but since only 100 eggs were used at each concentration the validity of the calculation must be in some doubt. The reconstituted water used in the tests had a hardness of about 100 mg/l as $CaCO_3$ and a pH of 7.2-7.8. For rainbow trout and goldfish teratic larvae were observed at exposure levels which did not affect egg hatchability significantly. The test was repeated with natural water at pH 7.8 and 174 mg $CaCO_3$/l hardness in which the LC50 for rainbow trout was 0.09 mg Ni/l. Dechlorinated tap water (pH 7.6, hardness 125 mg/l as $CaCO_3$) gave an LC50 of 0.06 mg Ni/l.

In the same quality of dilution water as that used by Birge *et al.* (1978), Birge and Black (1980) found that the LC50 from fertilization to four days post-hatch was 0.71 mg/l for channel catfich (*Ictalurus punctatus*) and 2.78 mg/l for goldfish (*Carassius auratus*). The LC10 values were 0.04 and 0.4 mg/l respectively. The paper also gave LC10 values for the embryo/larval stages of rainbow trout (0.01 mg/l) and largemouth bass (0.11 mg/l) derived from the earlier study.

2.4 Long-Term Effects

Apart from life-cycle tests which have already been described (Section 2.3) only two chronic studies have been performed. In the first, with 68-mm roach (*Rutilus rutilus*) in hard water (approx. 260 mg/l as $CaCO_3$) and at 13.7°C and pH 7.6 the 109-d LC50 was 3.4 mg/l (C.A. Willis, personal communication). In the second, bioaccumulation (Calamari, Gaggino and Pacchetti, 1982) and several biochemical parameters (Arillo *et al.*, 1982) were studied. Rainbow trout were exposed for 6 months to 1 mg Ni/l and to a mixture consisting of 0.01 mg Cd/l, 0.2 mg Cr/l and 1.0 mg Ni/l in hard water (320 mg/l as $CaCO_3$) at 15°C and a pH of 7.4. The treatment concentration was about 0.02 of the 48-h LC50 (see Section 2.1).

Metal-treated fish showed a significant depletion in sialic acid content of the gill and a marked decrease in total liver glucides in mature males which was also statistically significant in females.

Arillo *et al.*, (1982) concluded that long-term exposure to a concentration of 1.0 mg Ni/l and the mixture of nickel, chromium and cadmium caused several biochemical changes in *Salmo gairdneri*, some of which appeared to be irreversible, particularly in males.

2.5 Field Data

No data are available from studies in the field in which nickel occurs as the principal pollutant. The metal is normally found as a minor constituent together with other pollutants (see for example the study of Alabaster *et al.*, 1972). The contribution of nickel to any observed effect on the status of fisheries in these circumstances is uncertain. In 31 rivers in the UK the range of mean concentrations of dissolved nickel was from 0.012 to 0.073 mg/l for relatively polluted waters and from 0.0007 to 0.0037 for clean rivers (Mance and Yates, 1984).

3. ACCUMULATION IN FISH

3.1 Laboratory Data

Calamari, Gaggino and Pachetti (1982) found that in long-term exposure to 1 mg/l a continuous, uptake of nickel occurred for 180 days. For liver the concentration was around 2.9 mg/kg wet weight, for kidneys 4.0 and for muscle 0.8 mg/kg while, at the beginning of the experiment, the levels had been 1.5, 1.5 and 0.5 mg/kg respectively. They also found, by means of a toxicokinetic model, that theoretical asymptotic values for liver, kidney and muscle should be reached in 397, 313 and 460 days respectively at which times the calculated bioconcentration factors (BCF tissue conc/environmental conc) were 3.1, 4.2 and 1.0 respectively for the three organs. In clean water release was rather slow; the proportions of nickel remaining after 90 days were 25, 41 and 31% respectively for liver, kidney and muscle.

In conclusion, it has been shown from limited data that nickel has little capacity for accumulating in the tissues examined. However even these relatively low concentrations can cause biochemical damage (Arillo *et al.*, 1982) as summarised in Section 2.4.

3.2 Field Data

A review of the accumulation of nickel in aquatic organisms has been published recently (Jenkins, 1980). The reported range for uncontaminated areas in whole fish on a wet-weight basis was 0.2-2.0 mg/kg. Most of the field data were collected by North American authors and in general are not dissimilar. For example Uthe and Bligh (1971) found very low levels for Ni (0.2 mg/kg) in seven species of fish from contaminated and uncontaminated areas. Tong *et al.* (1972) gave data for several species of fish, decapitated and eviscerated; on a wet-weight basis values around 1 mg/kg were found with slightly higher concentrations in a few specimens with an overall range of 0.03 to 3.8 mg/kg. In contrast, Hutchinson *et al.* (1975) analysed rock bass (*Ambloplites rupestris*), white sucker (*Catostomus commersoni*), *Esox* sp., brown bullhead (*Ictalurus nebulosus*) and northern redhorse (*Moxostoma macrolepidotum*) from nickel-contaminated areas and found the following ranges of concentrations: 10.7-17.0 mg/kg in liver, 11.8-51.6 in kidney, 11.1-31.7 in gill and 9.5-13.3 in muscle. Mathis and Cummings (1973) found from 0.04 to 0.52 mg/kg in the muscle of several species of fish from Illinois River while Jeng (1975) in Asian waters found 0.3 mg/kg.

All these data are in agreement with the few analyses made by Gaggino (1983) on European fish from relatively clean water. This author analysed bleak (*Alburnus alburnus*) and chub (*Leuciscus cephalus*) from the River Po and found values from 0.15 to 0.27 mg Ni/kg for the first species and from 0.17 to 0.3 mg/kg for the second species in relatively uncontaminated areas, where concentrations of nickel in the water were about 0.003 mg/l. On the other hand in a contaminated sampling station the nickel content in the fish was 0.51 mg/kg for *Alburnus alburnus* and 0.75 mg/kg for *Leuciscus cephalus*.

4. EFFECTS ON FRESHWATER INVERTEBRATES

4.1 Introduction

Very few studies have been made in Europe on the effects of nickel on freshwater invertebrates. Even by drawing on non-European literature (largely North American) the data are too scarce to permit proper assessment of the influence of, for example, environmental factors on toxicity. No strict comparisons of toxicity to a given species at different temperatures, pH values, concentrations of dissolved oxygen or salinity are possible. Comparisons of sensitivity between species however can be made because many of the studies were carried out in water having hardness values around 50 mg/l as $CaCO_3$ and temperatures around 20°C.

4.2 pH and Alkalinity

Brkovic-Popovic and Popovic (1977) studied the effects of nickel and other heavy metals on the survival of *Tubifex tubifex* in four waters of different composition. The 24-h LC50 reduced from 120 mg Ni/l in hard water (pH 7.32, alkalinity 234 mg/l as $CaCO_3$) to 33.4 (28.1-39.7) mg/l in soft water at pH 6.85 and alkalinity 7.5 mg/l as $CaCO_3$. The corresponding 48-h LC50 values were 61 and 8.7 mg Ni/l.

4.3 Hardness

Information provided in the study described in 4.2 above can also be used to examine the effect of hardness on the toxicity of nickel to *Tubifex tubifex*. A decrease in hardness from 261 mg/l as $CaCO_3$ to 34.2 mg/l increased the toxicity of nickel so that the 48-h LC50s were 61 and 8.7 mg/l, as reported above. However, it should be noted that the effects of hardness, alkalinity and pH were not investigated independently, so only general trends should be read from the data.

4.4 Age of Organism

Although Anderson (1950) realized the importance of the age of *Daphnia* and, particularly, the sensitivity of the moulting period, the only study from which effects of age can be derived is that of Rehwoldt *et al.* (1973). They exposed the eggs and adults of the snail *Amnicola* to nickel in water having the following characteristics: temperature 17°C; pH 7.6; hardness 50 mg/l; dissolved oxygen 6.2 mg/l. Under these conditions the 24-h LC50s were 26.0 mg/l to eggs and 21.2 mg/l to adults while at 96 h this small difference in sensitivity was reversed, the 96-h LC50s being 11.4 and 14.3 mg/l, respectively.

4.5 Inter-Specific Differences in Lethal Toxicity

Table 1 sets out the LC50 values derived from short-term tests together with the test conditions. No longer-term data were found. Discounting the studies made in distilled water some of the smaller Crustacea (*Daphnia*, *Eudiaptomus* and *Nitocra*) appear to be most sensitive in the short term, with the rotifer *Philodina* and a mayfly (*Ephemerella*) slightly more tolerant. Nickel sulphate appeared less toxic to the rotifer than nickel chloride. Tubificid worms and chironomids, despite the known tolerance to organic pollution of certain members of these families, are next most sensitive (although in hard water the tubificids can be very much more resistant). Among the more tolerant groups of invertebrates are other Crustacea (*Gammarus* and *Cyclops*), oligochaetes (*Nais*), insects (Zygoptera), the Trichopoteran *Hydropsyche* and Plecoptaran (*Acroneuria*) and finally the molluscs *Amnicola* and, especially, *Viviparus*.

However, apart from *Daphnia*, the 96-h LC50s of the freshwater invertebrates studied, appear to be well in excess of 1 mg/l, even in waters with a hardness as low as 30 mg/l as $CaCO_3$.

4.6 Sub-Lethal Responses

Few quantitative data exist on the sub-lethal effects of nickel on invertebrates although information may be found on the accumulation of the metal in organisms (see Section 4.7, below).

Gupta, Khangarot and Durve (1981) found that *Viviparus bengalensis* reacted to high concentrations of nickel by closing the operculum and secreting or releasing mucus, eggs and embryos.

Biesinger and Christensen (1972) studied the survival, growth, reproduction and metabolism of *Daphnia magna*. The water-quality conditions of the test are set out in Table 1. *Daphnia* which had been exposed to 0.125 mg Ni/l for three weeks were 43% lower in weight than control *Daphnia*, with 9% less proteins, and glutamic oxalacetic transaminase activity reduced by 26%. Compared with the highest rate of reproduction (which might be found either in the controls or at very low levels of added metal - which of the two was not made clear for nickel), 16% impairment of reproduction occurred at 0.03 mg Ni/l with 50% impairment at 0.095 mg/l. These concentrations may be compared with the lethal concentrations given in Table 1.

Nickel can have effects on the growth, biochemistry and behaviour of invertebrates at levels which are significantly lower than lethal concentrations, but too few data are available to be more precise.

4.7 Accumulation

Hall (1982) exposed *Daphnia magna* to 0.25 mg/l nickel, including ^{63}Ni, at pH 6.9, DO 7-8 mg/l, and 18-21°C in water with a hardness of 60 mg/l as $CaCO_3$. The rate of uptake of nickel was quite rapid at first (to about 12 mg per animal within 20 h exposure) but then progressively slowed down, reaching about 24 mg in 80 h. Depuration also occurred, probably by means of two separate mechanisms, and 25-33% of the nickel was lost from the animal in the exuviae, shed on moulting. Gut tissue did not begin to accumulate nickel until after the first five hours of exposure, which suggested that food intake was not an important route of entry for nickel.

Cowgill (1976) reared *Daphnia magna* and *Daphnia pulex* for 3 months on *Euglena gracilis* which had been cultured in spring water containing 8.9×10^{-4} mg Ni/l. The algal cells contained 1.8 mg/kg, *Daphnia magna* 3.6 and *D. pulex* 4.2, giving BCF values of 2020, 4050 and 4720 respectively.

Few field studies have been made: Mathis and Cummings (1973) measured nickel in sediments, water and biota in the Illinois River. The water contained the lowest concentrations of nickel (<0.01 mg/l) and the sediments the highest (3 to 124 mg/kg). (Three non-industrial streams were also sampled and their sediments ranged from 10 to 22 mg/kg in nickel content.) Tubificid worms in the river were of two species - *Limnodrilus hoffmeisteri* and *Tubifex tubifex* and they contained 4 to 18 mg Ni/kg wet weight. Three species of clam were examined: in order of increasing nickel content (mg/kg on a wet-weight basis) they were *Quadrula quadrula* (0.4-1.6), *Amblema plicata* (0.4-2.3) and *Fusconaia flava* (0.7-3.0). Neither worms nor clams were starved before being examined and may therefore include metals in their gut contents.

Nickel may enter streams in the drainage from roads originating in the fuel and other material from traffic. Therefore streams crossed by roads carrying heavy traffic may receive more nickel than streams crossed by less-used roads. Van Hassel, Ney and Garling (1980) examined this possibility, and its consequences. They found that predatory stoneflies, of the family Perlidae, and detritivore Tipulids had a greater nickel content with increasing traffic density but Pteronarcid stoneflies (detritivores) did not. As traffic density rose from <50 vehicles/d to 15 000 vehicles/d concentrations in the Perlids rose from 2.9 to 11.0 mg Ni/kg weight and in Tipulids from 1.8 to 6.7 mg/kg. Samples of the water did not indicate much difference in the occurrence of nickel but the sediments increased from 0.9 to 1.6 mg/kg dry weight.

In summary, organisms can take up nickel from their environment until concentrations exceed those in their food, the surrounding water or sediments, depending on the particular route of uptake. There is a little evidence that crustacea may shed at least part of their nickel content on moulting and when placed in clean water.

4.8 Summary of Effects on Invertebrates

The concentrations of nickel causing mortality among invertebrates exposed for short periods (less than one week) were of the same order of magnitude as those affecting fish during such exposure. Only *Daphnia* species were killed at concentrations below 1 mg/l, as were certain young stages of sensitive species of fish (see Section 2.3, above).

The data presented in Table 1, suggest that for *Daphnia* 96-h LC50 values will be less than 0.5 mg/l. Rotifers, certain crustacea and mayflies exhibit 96-h LC50s in the range 1-5 mg/l while values for tubificids and chironomids were from 5 to 10 mg/l. Molluscs, *Gammarus*, *Nais*, stoneflies, damselflies and caddiflies were the least sensitive to nickel, 96-h LC50s often being much greater than 10 mg/l.

From the very few data little can be said about the effects of environmental factors on the toxicity of nickel to invertebrates except that an increase in hardness appears to reduce toxicity (as in Fish, Section 2.2.2, above).

Invertebrates can respond sub-lethally to the presence of nickel. Deleterious changes have been reported in growth rate, enzyme activity and reproductive rate of invertebrates exposed to sub-lethal concentrations of nickel, but the data are very limited.

Compared with the concentrations of nickel in the surrounding water invertebrates accumulate the metal, probably by direct uptake from the water as well as from their food or the sediments (in which the highest concentrations of nickel were generally found).

5. EFFECTS ON PLANTS AND MICRO-ORGANISMS

5.1 Algae and Macrophytes

5.1.1 Toxic effects

Hutchinson and Czyrska (1975) exposed *Lemna minor* (*valdiviana*) for three weeks to concentrations of nickel from 0.01 to 1.0 mg/l and found that 0.05 mg/l stimulated growth and only concentrations greater than 0.1 mg/l inhibited growth as compared with controls. The studies were conducted in an artificial medium at pH 6.8 and 24 $\pm$ 2°C in a photoperiod of 16 h light per day.

At 20°C a nickel concentration of 0.1 mg/l inhibited the growth of four species of green algae (*Pediastrum tetras*), *Ankistrodesmus falcatus* (and variety *acicularis*), *Scenedesmus quadricauda* and *S. dimorpha*) but 0.6 mg/l did not affect the blue-green *Anabaena cylindrica*, although it did reduce the rate of growth of *Anabaena flos-aquae* (Spencer and Greene, 1981). However, measurements of phytoplankton productivity in water from a shallow lake to which nickel nitrate was added indicated that the algae were unaffected even at 0.6 mg/l Ni. This is not surprising because (a) the community was dominated by blue-green algae and (b) although in the laboratory experiments using an artificial medium 98% of the nickel was in the form of the ion Ni^{2+}, less nickel may have been in a biologically available form in the lake water due to complexation with organic matter, including extracellular products of the blue-green algae.

Stratton and Corke (1979) found that 0.125 mg Ni/l inhibited growth of *Anabaena inequalis* but 10 mg/l was required to inhibit photosynthesis and 20 mg/l to inhibit nitrogenase activity.

Chiaudani and Vighi (1978) exposed *Selenastrum capricornutum* to nickel in a standard medium, but without EDTA. At 24°C and pH 6.0-6.3 the 7-d EC50 (inhibition of growth to 50% of control values) was 0.0025 mg/l as Ni. If 0.3 mg/l EDTA was added to the medium the 7-d EC50 of nickel was increased to 0.013 mg/l. In a subsequent experiment, similar to some of the work of Spencer and Greene (1981), tests were made in water from 22 lakes of various qualities from conductivity levels of 98 to 456 uS, alkalinities 100 to 440 mg/l as $CaCO_3$ and hardness values 55-260 mg/l. The addition of 0.04 mg Ni/l to filtered samples from these lakes did not result in as much inhibition of growth as might have been expected from the laboratory studies.

The diatom *Navicula pelliculosa* exposed to 0.1 mg/l nickel, of which all but 0.2% was said to be Ni^{2+}, exhibited retarded growth (50% of control value) during an exposure period of 14 days (Fezy, Spencer and Greene, 1979).

Algae may be able to acclimatise to the presence of nickel to some extent. Stokes (1975) examined *Scenedesmus acutiformis* var. *alternans* isolated from a lake in the mining and smelting area of Sudbury, Ontario. The lake water had contained about 2.5 mg Ni/l for the previous five years. At a concentration of 1.0 mg/l *Scenedesmus* grew at 53% of the rate of the controls and even at 3.0 mg/l the alga grew, albeit at only 18% of the rate of the algae in uncontaminated water. These results can be compared with those of Spencer and Greene (1981), above.

Table 1

The short-term lethal toxicity of nickel to invertebrates

Species	Temp. (°C)	Hardness (mg/l as $CaCO_3$)	Alkalinity (mg/l as $CaCO_3$)	pH	DO (mg/l)	LC50, as Ni Period (h)	LC50, as Ni Concn (mg/l)	Notes	References
ROTIFERA									
Philodina acuticornis	20 ± 1	-	54-67	7.4-7.8	-	24 48 96	7.4 4.05 2.75	($NiCl_2$) av. of two estimates of LC50	Buikema, Cairns and Sullivan (1974)
"	"	-	"	"	-	24 48 96	7.15 7.15 7.3	($NiSO_4$) av. of two estimates of LC50	"
OLIGOCHAETA									
Tubifex tubifex	20	~ 0.1	0.1	6.3	-	24 96	0.12 (0.091-0.16) 0.082 (0.063-0.11)		Brković-Popović and Popović (1977)
"	"	34.2	7.5	6.85	-	24 96	33.4 (28.1-39.7) 8.7 (7.84-9.65)		"
"	"	"	22.5	7.2	-	24 96	21.6 (18.1-25.7) 7.0 (6.78-8.46)		"
"	"	261	234	7.32	-	24 96	120 (111-130) 61.4 (57.9-65.1)		"
Nais	17	30	-	7.6	6.2	24 96	16.2 14.1		Rehwoldt *et al.* (1973)

Table 1 (continued)

Species	Temp. (°C)	Hardness (mg/l as $CaCO_3$)	Alkalinity (mg/l as $CaCO_3$)	pH	DO (mg/l)	LC50, as Ni Period (h)	LC50, as Ni Concn (mg/l)	Notes	References
GASTROPODA									
Viviparus bengalensis	27.3	180	130	7.4	6.8	12 24 48 72 96	154 138 100 59 39.8		Gupta, Khangarot and Durve (1981)
Amnicola	17	30	-	7.6	6.2	24 96 24 96	26.8 11.4 21.2 14.3	eggs " adults "	Rehwoldt, *et al.* (1973)
INSECTA									
Trichopteran	17	30	-	"		24 96	48.4 30.2		"
Zygopteran	17	30	-	"		24 96	26.4 21.2		"
Chironomus	17	30	-	"		24 96	10.2 8.6		"
Acroneuria lycorias	18.5	40	54	7.0	8.0	96	33.5	Slight changes in quality during test	Warnick and Bell (1969)
Ephemerella subvaria	"	42	46	"	"	96	4.0	Slight changes in quality during test	"

Table 1 (continued)

Species	Temp. (°C)	Hardness (mg/l as $CaCO_3$)	Akalinity (mg/l as $CaCO_3$)	pH	DO (mg/l)	LC50, as Ni Period (h)	LC50, as Ni Concn (mg/l)	Notes	References
Hydropsyche betteni	18.5	48	42	7.0	8.0	336	>64.0	Slight changes in quality during test	Warnick and Bell (1969)
CRUSTACEA									
Gammarus	17	30	-	7.6	6.2	24 96	15.2 13		Rehwoldt, *et al.* (1973)
Nitocra spinipes	20 ± 5	-	15	8.0	-	48	6.0 (3.9-9.1)	Salinity 7°/oo	Bengtsson (1978)
Cyclops abyssorum prealpinus	10	-	58	7.2	-	48	15 (8.8-25.5)		Baudouin and Scoppa (1974)
Eudiaptomus padanus padanus	"	-	"	"	-	48	3.6 (2.8-4.6)		"
Daphnia hyalina	"	-	"	"	-	48	1.9 (1.45-2.48)		"
Daphnia magna	25	-	97-100[a/]	8.2-8.4[a/]	-	64	<0.32		Anderson (1950)
"	18 ± 1	45.3	42.3	7.74	-	48 48 504	0.51 1.12 0.13 (0.098-0.173)	Unfed Fed	Biesinger and Christensen (1972)

a/ Anderson (1944)

5.1.2 Accumulation

Clark *et al.* (1981) studied the accumulation and depuration of (*inter alia*) nickel by *Lemna perpusilla*. Plants collected from a fly-ash basin were allowed to depurate in dechlorinated tap water at 20°C for 14 days before laboratory experiments began. Accumulation was then examined over a 10-day period and depuration over the following eight days. During the first 14 days concentrations of nickel in the plants fell from about 160 mg/kg dry weight to about half this value and, in clean water, remained at this level. *Lemna* accumulated nickel readily, particularly at the lowest ambient concentration of 0.1 mg/l, reaching 500-600 mg/kg in the 10 days. A return to depuration conditions did not immediately result in a fall in the nickel content of the plants, but after eight days it was down to <160 mg/kg again. Since the concentration of nickel in the water of the ash basin was also 0.1 mg/l, greater accumulation occurred in the laboratory than in the field.

Cowgill (1976) found that *Euglena gracilis*, exposed to 8.9×10^{-4} mg Ni/l in spring water accumulated the metal to a concentration of 1.8 mg/kg, a BCF of about 2 000.

"Kangkong air" (*Ipomea aquatica*) took up 200 mg Ni/kg dry plant in 48 h, mostly into the roots, from water containing 5 mg Ni/l (Low and Lee, 1981). The presence of 5 mg Cu/l slightly reduced the uptake and when copper and chromium were present at 5 mg/l uptake of nickel was less than half the value found with nickel alone.

Hutchinson and Czyrska (1975) examined *Lemna minor* from 23 sites which had a mean concentration of nickel in the water of 0.027 mg/l. The plants contained from 5.4 to 35.1 mg Ni/kg (dry weight), equivalent to concentration factors of 200 to 1 300. They also grew *Lemna* on a culture medium at pH 6.8 and 24 ± 2°C for 3 weeks with nickel (0.01 to 1 mg/l), and in some cases nickel and copper, added to the medium; accumulated nickel concentrations ranged from 40 mg/kg dry weight at 0.01 mg/l to 3 067 mg/kg at 0.5 mg/l. The presence of copper slightly increased the accumulation of nickel by *Lemna*, unlike the decrease found with *Ipomea*, above.

Stokes (1975) also found a copper-enhanced uptake of nickel in the alga *Scenedesmus*, obtained from a lake in which concentrations of copper and nickel had averaged 0.5 and 2.5 mg/l respectively for the previous five years. She suggested that the greater uptake of nickel was a result of copper increasing the permeability of the cell membrane.

Other data can be found in the review by the National Research Council of Canada (1981) giving data on other freshwater macrophytes, sometimes divided into roots, shoots, stems and leaves. Values ranged from a few to several hundred mg Ni/kg.

5.2 Fungi and Micro-Organisms

Babich and Stotzky (1982) reviewed the effects of nickel on micro-organisms and discussed the effect of pH on toxicity. Filamentous fungi varied considerably in their response to nickel, the growth of some being inhibited by 10 mg Ni/l and others being affected only by concentrations as high as 500 mg/l. The colonies sometimes responded by changes in form and coloration. Responses to changes in pH varied between species but an increase in pH frequently caused a decrease in toxicity.

With eubacteria and actinomycetes there was less variability in toxicity and the range of concentrations inhibiting growth was generally 5 to 30 mg/l Ni, although some managed to grow at 100 mg/l. Reductions in pH increased toxicity. With yeasts, the range of inhibitory concentrations was 1 to 40 mg/l and a change in pH affected toxicity in some cases, as above.

In a later paper Babich and Stotzky (1983) investigated the influence of various environmental factors on the toxicity of nickel to, among other organisms, an actinomycete, four species of eubacteria and a yeast. Reductions in cell number occurred at 5 or 10 mg/l and viable cells were eliminated at 10 to 50 mg/l, although some species were unaffected after 24 h at 100 mg/l. Reductions in pH from 6.8 to 5.3 enhanced the toxicity of 75 mg Ni/l to some species but not to others. In harder water there was some reduction in toxicity of nickel, and the addition of 50 mg/l of a clay mineral or mixed organic and inorganic particles also reduced toxicity. It seemed that the metal was absorbed on the particles.

Bringmann, Kuhn and Winter (1980) found that in a fairly hard water (approx. 150 mg/l as $CaCO_3$) and at a pH of 6.9 the saprozoic flagellate *Chilomonas paramecium* reduced in number at 0.82 mg Ni/l.

5.3 Summary of Effects on Plants and Micro-Organisms

Very little work on the toxic effects of nickel on macrophytes has been carried out. A concentration of 0.1 mg/l harmed *Lemna* but green algae were affected at much lower concentrations, even as low as 0.0025 mg/l (*Selenastrum*). However, this latter result was obtained in laboratory cultures in the absence of chelating agents. In other work, concentrations of 0.04 mg Ni/l added to natural waters appear to have been only marginally damaging. Adaptation to high concentrations of nickel seems to produce resistant strains in some green algae. Apart from these considerations it is common to find reports of inhibition of green algae at around 0.1 mg Ni/l.

Blue-green algae are noticeably more resistant and eubacteria are even more tolerant. Growth of species of *Anabaena* has been found to be inhibited at 0.6 mg/l; sensitive yeasts may be harmed at 1 mg/l but only the most sensitive enbacteria are affected at 5 mg/l. Filamentous fungi are seldom harmed below 10 mg/l.

The toxicity of nickel to plants can be reduced in the presence of organic complexes, while increases in pH or hardness and organic and inorganic solids can reduce the toxicity of the metal to micro-organisms.

6. SUMMARY AND CONCLUSIONS

Nickel occurs widely in nature, particularly in basic rocks (1.1). The sulphide ores are often exploited, the metal being used in electro-plating and the production of alloys. Nickel is released into the environment during mining, smelting, manufacture and the combustion of fossil fuels.

The predominant oxidation state of nickel in natural waters is the divalent cation, Ni^{2+}, and in waters of pH 5 to 9 this ion is almost the only form present. Complexes of naturally occurring ligands are formed to a small degree. Many stable complexes occur with organic ligands and the metal can also be adsorbed onto clay minerals. The contribution to toxicity of these two last forms is difficult to predict (1.2). Nickel concentrations in natural waters (1.4) vary from 0.001 to 0.020 mg/l with most measurements being below 0.005 mg/l.

No precise descriptions of the biochemical basis of the toxicity of nickel have been made, but the metal is known to bind to a variety of molecular structures such as nucleic acids and proteins; effects on several enzymes have been reported. Nickel induces hypoglycaemia in mammals, and fish exposed to nickel showed a marked drop in glucidic stores. This effect could be attributed to direct metal interaction on both membranes and enzyme thiolic groups of the pancreatic cells.

Other effects, similar to those found with other metals, such as damage to secondary lamellae of gills and sialic acid depletion in gills have been described (2.1).

In short-term tests in soft water the most sensitive species of freshwater fish are killed by exposure to concentrations of about 4 to 20 mg Ni/l. Higher LC50 values of nickel to different species of fish have been found in harder waters, from about 30 to 80 mg Ni/l. From the limited data available it appeared that hardness had the greatest effect on toxicity while other determinants have not been proved to have a significant effect. In acute tests interspecific differences occurred in sensitivity, but were ranged within a single order of magnitude (2.2).

The acute lethal toxicity of mixtures which include nickel can in general be predicted on the basis of additive joint action.

Juvenile rainbow trout are less sensitive than alevins, for which the 12-d LC50 in hard water is 5 mg/l while in another study the 56-d LC50 was found to be 1.3 mg/l.

In a life-cycle study in a moderately hard water (200 mg/l as $CaCO_3$) a concentration of nickel of 0.38 mg/l caused no adverse effect on the survival, growth and reproduction of fathead minnow. The 10-d LC50 nickel to carp larvae was 0.75 mg/l in water having a hardness of 100 mg/l as $CaCO_3$. In water with this hardness the LC50s to fish, exposed from fertilization to 4 days post-hatching, were in the range 0.05-2.78 mg/l according to species, rainbow trout being the most sensitive (2.3). The LC10 to rainbow trout under these conditions was about 0.01 mg/l.

Long-term exposure of rainbow trout to 1 mg/l nickel in hard water caused several biochemical alterations, some of which appear to be irreversible (2.4).

Laboratory studies have shown that nickel has little capacity for accumulation in all the fish studied (3.1). However it has been demonstrated that this relatively low concentration of nickel in tissues could cause biochemical damage (2.4). The range of concentrations reported for uncontaminated areas in whole fish on a wet-weight basis are 0.2-2 mg/kg. This value could be enhanced by a maximum of tenfold in contaminated areas (3.2).

The concentrations of nickel causing mortality among invertebrates exposed for short periods (less than one week) were of the same order of magnitude as those affecting fish during such exposure, except that, for species of *Daphnia*, toxic concentrations are less than 0.5 mg/l in 96-h periods of exposure (4.5).

A concentration of 0.1 mg/l harmed the macrophyte *Lemna* but green algae were affected at much lower concentrations (5.1). However, concentrations as low as 0.04 mg/l, added to natural waters, appear to have been only marginally damaging.

Fungi and micro-organisms (5.2) demonstrate a fairly wide variety of sensitivity to nickel but are generally more tolerant than the higher organisms mentioned above.

7. TENTATIVE WATER-QUALITY CRITERIA

From the data available no differentiation between proposed standards for salmonid and non-salmonid fish was considered justified. The concentrations given below are considered to be satisfactory also for the protection of the majority of other freshwater organisms. The average concentrations of "soluble" nickel should not exceed 0.01 mg/l and the 95 percentile should not exceed 0.03 mg/l in soft water (20 mg/l as $CaCO_3$). In hard water (320 mg/l as $CaCO_3$) it is proposed that the corresponding concentrations of nickel should be 0.04 mg/l and 0.12 mg/l. However there is evidence that higher concentrations of nickel might be satisfactory where organic complexing agents are present.

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