



**Centre for Environmental Contaminants Research  
Energy Technology**

**FIELD AND EXPERIMENTAL  
RESPONSES OF BIOTA TO  
COPPER-ENRICHED AND ACIDIC  
WATERS: LITERATURE REVIEW**

**by A.A. Chariton and S.C. Apte**

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(OTML)**

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## EXECUTIVE SUMMARY

A scoping literature review was conducted to evaluate the availability of field data on the effects of copper contamination and acid rock drainage (ARD) on aquatic biota. Particular emphasis was placed on algae, copper and the effects of aquatic ecosystem acidification. The review indicated that copper concentration below 2 µg/L have no observable effect on the composition, diversity and abundance of biota (algae, benthos and fish). Furthermore, significant recovery of formerly impacted invertebrate and fish communities has been shown to occur in environments subsequent to copper concentrations returning to below 2 µg/L. At slightly higher concentrations, the effects of copper become less defined. Changes in algal assemblages, including the dominance of taxa, have been shown to occur in the field above 3.7 µg/L. A reduction in biomass has been observed, both in mesocosm experiments and in the field, at concentrations ranging between 10-18 µg Cu/L. As copper concentrations increase, significant changes in the dominance of the taxa occur in both algal and biofilm communities. Generally, algal communities become dominated by copper-tolerant diatom taxa, with these taxa being more copper-tolerant than phytoplankton. As the conditions exceed the tolerance of diatoms, the communities shift towards single-cell green algae and cyanobacteria dominated communities. It is fairly clear that copper concentrations exceeding 150 µg/L have a significant effect on both filamentous and planktonic freshwater algae, with concentrations in this range being used as algicides.

A reduction in pH from alkaline conditions can have a pronounced effect on all main biotic groups. Below a pH of 7, decreases in mollusc populations are frequently observed. The limiting factor is the formation of their calcium carbonate shells under acidic conditions. Below a pH of 6.2, an imbalance in the ratio of algae production to respiration can occur, as can a decline in some algal species, often resulting in a dominance of acid-tolerant diatoms. Nitrification in lake systems has been shown to effectively cease at pH values between 5.6 and 6.5. For most anadromous fish species, a pH below 6 exceeds their physiological limitations, providing a relatively clear threshold for acceptable water. Below pH 5, sub-lethal and acute responses can be expected in a majority of benthos and algae, with dissolved aluminium being the most important toxicant. Below a pH of 3, acidity is considered to be the dominant stressor in modified aquatic systems, even in the presence of elevated metal loads.

## TABLE OF CONTENTS

EXECUTIVE SUMMARY .....	ii
TABLE OF CONTENTS.....	iii
1 INTRODUCTION .....	1
2 METHODS .....	2
3 RESULTS .....	3
4 DISCUSSION.....	5
5 CONCLUSION .....	7
6 REFERENCES .....	8

### List of Tables

TABLE 1. RESPONSES OF BIOTA TO INCREASED ACIDITY IN FORMERLY ALKALINE WATER BODIES.....	4
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### List of Appendices

APPENDIX A RESPONSE OF BIOTA TO COPPER CONCENTRATIONS BETWEEN 1-2 MG/L. * DENOTES STUDIES EXAMINING THE ECOLOGICAL RESPONSES AT SPECIFIC MINES OR ARD AFFECTED REGIONS.....	12
APPENDIX B RESPONSE OF BIOTA TO COPPER CONCENTRATIONS BETWEEN 2-10 MG/L.* DENOTES STUDIES EXAMINING THE ECOLOGICAL RESPONSES AT SPECIFIC MINES OR ARD AFFECTED REGIONS.....	13
APPENDIX C RESPONSE OF BIOTA TO COPPER CONCENTRATIONS BETWEEN 20-50 MG/L . *DENOTES STUDIES EXAMINING THE ECOLOGICAL RESPONSES AT SPECIFIC MINES OR ARD AFFECTED REGIONS .....	15

APPENDIX D RESPONSE OF BIOTA TO COPPER CONCENTRATIONS BETWEEN  
50-150 MG/L.\* DENOTES STUDIES EXAMINING THE ECOLOGICAL  
RESPONSES AT SPECIFIC MINES OR ARD AFFECTED REGIONS. .... 16

APPENDIX E RESPONSE OF BIOTA TO COPPER CONCENTRATIONS >150  
MG/L\* DENOTES STUDIES EXAMINING THE ECOLOGICAL RESPONSES AT  
SPECIFIC MINES OR ARD AFFECTED REGIONS. .... 17

## 1 INTRODUCTION

As part of Ok Tedi Mining Limited's (OTML) current risk assessment of various riverine tailings management options, there is a need to understand the effects of both copper and acid-rock drainage (ARD) on the biological communities of the Fly River. This is not an easy task and represents frontier science. Ecotoxicological studies conducted under controlled conditions with cultured organisms quite clearly show that many organisms are sensitive to dissolved copper at low  $\mu\text{g/L}$  concentrations (Rogers et al., 2005). However, the difficulty lies in extrapolating this laboratory-based toxicity information to the response of complex aquatic communities such as those found in the Fly River. In particular, effects such as ecosystem recovery, depletion of food sources, prey switching, and algal succession are not considered in ecotoxicological studies. Assessment using laboratory-based toxicity testing alone is likely to overestimate the importance of copper toxicity to individual organisms and underestimate the more subtle consequences of the ecosystem processes noted above.

Within the time bounds of the OTML risk assessment, it is not possible to conduct the detailed mechanistic studies required to unravel the effects of copper and acidity on biological communities. In a recent risk assessment workshop held in Sydney in July 2005, it was suggested that some useful information may be found in the literature covering systems already impacted by copper and ARD. This information may be used in a weight-of-evidence approach to gain a better appreciation of the effects of contaminants on aquatic communities of the Fly River.

A short scoping was conducted in order to assess the availability and usefulness of published data. Particular emphasis was placed on algae and their responses to copper and ARD.

## 2 METHODS

Using a variety of scientific databases, over 120 papers were collected encompassing the effects of ARD, copper and pH on biota. Specific mines and mining areas which were initially examined during this review included: Mount Lyell, Tasmania; Captains Flat, NSW; Mount Morgan, Queensland; Rum Jungle, NT; Mount Lofty, South Australia; Blackbird Mine, Idaho; Clark Fork River, Montana; Coeur d'Alene River Basin, Idaho; Upper Powell River Watershed, Virginia; East Prong Creek, Virginia; Clinch River, Virginia; Eagle River Colorado; Le An River, China; Ogoya Mine, Japan; Lake Orta, Italy; and Guadiamar River, Spain. After perusal, the collection was further filtered to include only those papers which were derived from field observations, mesocosm studies, large aquaria experiments utilising field collected biota, or relevant review articles. Papers were only selected if copper was identified as a major stressor, and where copper concentrations within the waters were presented. It should be noted that several well-known copper mines and ARD impacted locations were negated from this review, e.g. Mount Morgan. This was primarily due to three factors: (i) unavailability of data; (ii) the data was in format which did not permit direct comparisons between aqueous metal concentrations and biological responses; or (iii) only laboratory derived toxicity data was available.

This resulted in 39 papers primarily derived from freshwater studies (see references). Additional information regarding the concentration of copper for algicidal purposes was also obtained from several algicide manufacturers. For pH, papers were collected which specifically focused on comparisons and the manipulation of freshwater alkaline water bodies to acidic conditions.

It is emphasised that a very coarse approach was applied for assessing the effects of copper and changes in pH. In gradient studies, biological effects were extrapolated from the results in which copper appeared to be a dominant discriminating factor. In such studies, the aim was to identify copper concentrations at which both no observable effects occurred, and the concentrations where an effect was identified. In population studies, only lethal effects were utilised, whilst in the community studies, various indices and community metrics were recorded. Extrapolations of the findings from experimental studies were less ambiguous due to comparisons between controls and experimental treatments.

The identified copper-related papers were collated to examine the effects of copper on algae, benthos and fish across several concentration ranges (1-2, 2-10, 10-50, 50-150 and >150 Cu µg/L). Responses to biota were also classed according to a decline in pH. It should be noted that the reviewed studies do not take into account copper speciation. The dissolved copper concentrations quoted below for natural waters or natural waters amended with copper include non-bioavailable forms of copper. These reported values for natural waters should therefore be compared against typical dissolved copper concentrations in the Fly River. For studies utilising synthetic waters amended with copper,

concentrations should be compared to labile copper concentrations in the Fly River.

### **3 RESULTS**

#### **1-2 µg Cu/L**

There is no evidence from field studies and outdoor aquaria studies to suggest that copper concentrations between 1-2 µg/L pose any ecological threat to algal, invertebrate or fish communities (Appendix 1). Field studies examining the recovery of invertebrate and fish communities have found extensive recoveries in environments when copper concentrations fell within this range.

#### **2-10 µg Cu/L**

Declines in the richness, evenness and a shift in the dominance of algae taxa have been shown to occur in the field at concentrations as low as 1.9 µg Cu/L (Appendix 2). Experimental evidence indicates that changes in phytoplankton can occur as low 5 µg Cu/L, with significant changes in biomass and abundance occurring at 10 µg/L.

Gradient studies have found significant impacts on benthic communities occurring at concentrations  $\approx$  2.5 µg Cu/L; however, significant concentrations of other metals (e.g. zinc) were also present (Norris, 1986). Conversely, similar concentrations of copper coupled with zinc had no observed effect on benthos in experimental streams (Hickey and Golding, 2002). Experimental evidence has shown a significant impact of dissolved copper on the composition of benthos at 5 µg/L, with a similar concentration resulting in a reduction in the abundance of juvenile coleopterans in the field. Modelled estuarine and marine data indicate that concentrations exceeding 4.5 µg/L pose a significant effect on benthos, with fish susceptible to concentrations exceeding 5.6 µg/L. No observed effect was detected on caged juvenile salmon in estuarine waters exposed to 5 µg Cu/L.

#### **10-50 µg Cu/L**

Concentrations between 30-50 µg Cu/L appear to have a significant deleterious effect on algal and invertebrate communities, causing declines in the density and diversity of both fresh and marine algae, and marine invertebrates (Appendix 3). Exposure of caged snails to waters with 42 µg Cu/L resulted in 100% mortality.

#### **50-150 µg Cu/L**

Algae are still regularly observed in water having dissolved copper concentrations between 50-150 µg/L; however, significant alterations to the composition and dominance of species have been demonstrated both in the field and experimentally (Appendix 4). Within this range, a decline in diatoms appears

to occur, with a shift towards green algae and cyanobacteria dominated communities.

### 150 µg Cu/L

At concentrations exceeding 150 µg Cu/L, fresh and marine algal communities are significantly impaired or cease to occur (Appendix 5). However, the presence of some persistent unicellular algal species has still been observed in outdoor experiments. The use of copper as an effective algicide starts at a concentration of 150 µg/L (pH 7.2-7.4), although algicidal concentrations generally exceed 200 µg/L. Significant declines in the abundance of salmon fry have been observed at 160 µg Cu/L. Catastrophic responses in benthos have been observed at concentrations exceeding 260 µg Cu/L resulting in the complete loss of fauna.

### pH

A decrease in the pH below 7 in naturally alkaline water bodies can result in a decline of mollusc populations (Table 1). Below pH 6.2, dysfunction of lake metabolism has been demonstrated, with unbalanced production to respiration ratios. Below pH 6, waters become toxic to many anadromous fish, inhibiting of the formation of calcium carbonate shells in a majority of molluscs. Between pH 5.6 and 6.5 algal species richness has been shown to decline, with nitrification ceasing between pH 5.4 and 5.7. At pH 4-5, sub-lethal and acute responses occur in most species, with the response dependant upon aluminium concentrations. At pH 3, acidity is considered to be the primary stressor over-riding metal related effects.

**Table 1. Responses of biota to increased acidity in formerly alkaline water bodies**

pH	Response
7.0	Decrease in the abundance of molluscs
6.2	Decrease in the net production of periphyton, and unbalanced production:respiration
6.0	Toxic to most anadromous fish
6.0	Rare observance of molluscs
5.6- 6.5	Reduction in number of algal species
5.4 -5.7	Nitrification ceased in two experimentally acidified lakes.
5.0	Acute toxicity to most species when Al is present 20-50 µg/L, chronic toxicity when Al is 10-20 µg/L
4-5	Lethal to many species of invertebrates. Sub-lethal effects on egg development, hatching and larval growth in fish and invertebrates
3	Acidity becomes the primary stressor, over-riding the effects of metal toxicity

## 4 DISCUSSION

The results of the literature review indicate that copper concentration below 2 µg/L have no observable effects on the composition of biota (algae, benthos and fish). Furthermore, significant recovery of formerly impacted invertebrate and fish communities has been shown to occur in environments subsequent to copper concentrations returning to below 2 µg/L (Schultheis et al., 1997; Jeffree et al., 2001).

At slightly higher concentrations, the effects of copper become less defined. Changes in algal assemblages, including the dominance of taxa, have been shown to occur in the field above 3.7 µg/L and experimentally after 5 µg/L (Gustavson and Wandberg, 1995). A reduction in biomass has been observed at concentrations ranging between 10-18 µg/L, both experimentally and in the field. Although algal biomass does increase in some incidences when exposed to acid mining, diversity generally declines (Niyogi et al., 2002).

As copper concentrations increase, significant changes in the dominance of the taxa in both algal and biofilm communities occur. Generally, algal communities become dominated by copper-tolerant diatom taxa, with these taxa being more copper-tolerant than phytoplankton. As the conditions exceed the tolerance of diatoms, the communities shift towards single-cell green algae and cyanobacteria dominated communities. For example, Soldo and Behra (2000) found Chlorophyta (green algae) to be unaffected in experimental treatments of 64 µg/L, whilst Barranguet et al. (2003) observed a shift towards cyanobacterially-dominant biofilms in experimental streams with concentrations exceeding 76 µg/L. Similarly, Havens (1994) reported a decline in diatom biomass concurrent with an increase in bacterial biomass at 140 µg/L. The increase in bacterial biomass may also be an indirect response to the increase in nutrients following the decomposition of decaying algae.

It is fairly clear that copper concentrations exceeding 150 µg/L have a significant effect on both filamentous and planktonic freshwater algae, with concentrations in this range being used as algicides. However, in a 12-day outdoor experiment, algal communities were still observed in treatments exposed to 318 µg/L, although periphyton abundance and diversity was significantly reduced, with unicellular green algae becoming the dominant taxa (Soldo and Behra, 2000).

Low to intermediate concentrations of copper produced a diverse range of responses in benthos, as they did with algae. For example, Norris (1988) found benthic communities to be impaired by former mining activities at dissolved copper concentrations of 2.5 µg/L, although increases in other metals and changes in water chemistry also occurred. Conversely, a mesocosm study at similar copper and zinc concentrations showed no significant effect on benthic assemblages (Hickey and Golding, 2002). Copper appears to have an effect on the structure and function of benthic communities at concentrations exceeding µg Cu/L, with a marked effect clearly evident from both experimental and field data at concentrations exceeding 12 µg Cu/L. In some instances, this may result in a

reduction of herbivorous taxa with concurrent increases in some predator species, including some chironomids (e.g. Hickey and Golding, 2002). At greater concentrations ( $>30 \mu\text{g/L}$ ), copper has a significant impact on benthos, with impoverished communities being observed in the field ( $30 \mu\text{g/L}$ ), and 100 percent mortality occurring in snails placed *in situ* (Reed and Gadd, 1990; Watanabe et al., 2000). Field observations have found concentrations of 260 and  $500 \mu\text{g Cu/L}$  to have a catastrophic effect, resulting in the complete loss of benthic fauna (Okada and Uesugi, 1958 cited in Watanabe et al., 2000; David, 2003).

The limited *in situ* information available on the effect of copper on fish makes it difficult to draw any assumptions regarding field threshold values for no observable effects. A significant recovery of fish populations in the Rum Jungle uranium/copper mine occurred at sites where remedial practices resulted in  $< 5 \mu\text{g Cu/L}$ . Similar concentrations have shown no deleterious effect on the survival of caged juvenile salmon (Barry et al., 2000). Modeled data from European estuarine and marine environments suggests that concentrations exceeding  $5.6 \mu\text{g/L}$  are potentially toxic to fish. At concentrations exceeding  $120 \mu\text{g Cu/L}$ , significant impacts on fish communities have been observed, with the abundance of salmon fry significantly attenuated at  $166 \mu\text{g Cu/L}$ .

A reduction in pH from alkaline conditions can have a pronounced effect on all main biotic groups. Below a pH of 7, decreases in mollusc populations are frequently observed in Australia, with an absence of mollusc occurring in most water bodies below pH 6 (R. Norris, University of Canberra, personal communication). The limiting factor is the formation of calcium carbonate shells under acidic conditions. Below a pH of 6.2, an imbalance in the ratio between algae production and respiration can occur, as can a decline in some algal species, often resulting in a dominance of acid-tolerant diatoms (Schindler, 1990). In a long-term study examining the acidification of two experimental lakes, Schindler (1990) found nitrification to effectively cease at pH values between 6.5 - 5.6. For most anadromous fish species, a pH below 6 exceeds their physiological limitations, providing a relatively clear threshold for acceptable water (Fromm, 1980). Below pH 5, sub-lethal and acute responses can be expected in a majority of benthos and algae, with concurrent concentrations of aluminium influencing the magnitude of any likely effect on benthos (Ledger and Hildrew, 2005). However, the some taxa are found in high abundances under these conditions, e.g. some carnivorous chironomids (Soucek, 2001; Janssens de Bisthoven et al., 2005). Several studies have shown found benthic communities to be severely impoverished at river sites with  $\text{pH} \approx 4$  (Jarvis and Younger, 1997; Anon, 1999). Below a pH of 3, acidity is considered to be the dominant stressor in modified aquatic systems, even in the presence of elevated metal loads.

Changes in the acidity of the waters can also have an indirect effect on algal communities through attenuating the concentrations of nutrients required to support primary production. For example, a reduction in pH can reduce the

concentration of dissolved organic carbon and phosphorus. This can limit algal growth, creating oligotrophic conditions in former eutrophic systems.

## **5 CONCLUSION**

Although there is a high level of variability between studies, the results of the literature review suggest that biotic responses in algae and benthos are initiated at concentrations exceeding 5 µg Cu/L, and become significantly more pronounced at concentrations of 10-15 µg Cu/L. For algae, this predominantly involves a decline in phytoplankton prior to any major changes in phyto-benthos, with the magnitude of the impact being dependent upon many factors, including exposure history, species, density and water chemistry. Similar concentration responses appear to occur in benthos; again, shifts in the dominance of specific taxa and trophic levels (e.g. collectors to predators) provide a precursor for significant alterations to assemblages.

The difficulty resides in extrapolating these findings to the resources required to support healthy fish populations. In order to do this, the specific requirements of the fish need to be understood. More specifically, if sensitive algae and benthos are reduced or eliminated, will this have trophic ramifications along the food web? The effect on the system is dependent on the niche overlaps and trophic interactions of component species. For example, in a system where particular species are considered functionally similar, a reduction in species richness may not result in a loss of function if the biomass of the group is maintained. Conversely, the loss of a species that drives discrete processes in communities could lead to functional impairment, irrespective of shifts in biomass.

The concentrations provided within this review may be conservative, as a number of the studies included several co-stressors; however, it should be recognised that seasonal changes can add additional pressures to fish communities, most notably, low dissolved oxygen during some periods. If fish populations are being marginally maintained due to acid mining, a pronounced effect (e.g. increased seasonal fish kills) can be expected when sub-optimal conditions are compounded with mining impacts. Consequently, copper concentrations should be approached conservatively to reduce the likelihood of such events.

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**Appendix A Response of biota to copper concentrations between 1-2 µg/L. \* denotes studies examining the ecological responses at specific mines or ARD affected regions.**

Type of Biota	Dissolved Cu (µg/L)	Observed effect	Type of study	Reference
Algae	1	NOE in phytoplankton	Outdoor aquaria (20days)	Gustavson and Wandberg, 1995)
	1.3	NOE in algae*	Gradient – Le An River, China	He et al., 1998
Invertebrates	1	NOE in zooplankton	Outdoor aquaria (20days)	Gustavson and Wandberg, 1995
	1.1	NOE in benthos*	Field- Sudbury smelters, Canada	Iles and Rasmussen, 2005
	1-2	Most taxa similar to controls*	Recovery in field- East Prong Creek, Virginia	Schultheis et al., 1997
	1.3	NOE on benthos*	Gradient - Le An River, China	He et al., 1998
Fish	1-5	Recovery of fish communities*	Recovery in field - Rum Jungle, NT.	Jeffree et al., 2001

NOE: No observable effect

**Appendix B Response of biota to copper concentrations between 2-10 µg/L.\* denotes studies examining the ecological responses at specific mines or ARD affected regions.**

Type of Biota	Dissolved Cu (µg/L)	Observed effect	Type of study	Reference
Algae	1.9-8.9	Decline in algae species*	Gradient- Le An River, China	He et al., 1998
	3.7-11.8	Decrease in diatom diversity and change in dominant taxa*	Gradient - Guadiamar River, Spain	Sabater, 2000
	5	Changes in phytobenthos structure	Outdoor aquaria (20days)	Gustavson and Wandberg, 1995
	6.4	A loss of a few species: tolerance increased by pre-exposure.	Outdoor (12 day)	Soldo and Behra, 2000
	10	Reduced algae biomass and abundance	Large aquaria and field	Gausch et al., 2002
	14.7	Change in distribution of algae*	Gradient – Dexing Cu Mine, China	Liu et al., 2003
	15	Decrease in phytoplankton abundance, diversity and biomass	outdoor aquaria (20days)	Gustavson and Wandberg, 1995
	18	Decline in periphyton biomass*	Gradient- Eagle River, Colorado	Hill et al., 2000
Invertebrates	2.5	Loss of many taxa*	Field gradient – Captains Flat, NSW	Norris, 1986
	2.5	NOE on community indices except an increase in taxa	Mescosm (includes Zn)	Hickey and Golding, 2002

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		richness		
	4.5	Risk to estuarine benthos	Modelled field data	Hall and Anderson, 1999
	5	Decline in many taxa	Experimental stream	Leland et al., 1989
	5	Reduced abundance of juvenile coleoptera*	Field – Blackbird Mine, Idaho	Beltman et al., 1999
	12	Significant effect on benthos communities*	Field – Blackbird Mine, Idaho	Beltman et al., 1999
	12	Significant impact on benthos	Modelled field data	Sloane and Norris, 2003
	12	Significant impact of benthos composition*	Recovery in field- East Prong Creek, Virginia	Schultheis et al., 1997
	13	Significant impact on benthos	Mesocosm (includes Zn)	Hickey and Golding, 2002
	14	Loss and reductions in many taxa*	Field- Sudbury smelters, Canada	Iles and Rasmussen, 2005
Fish	5	NOE on caged juvenile salmon*	Field – Howe Sound, Canada	Barry et al., 2000
	5.6	Risk to estuarine and marine fish.	Modelled field data.	Hall and Anderson, 1999

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NOE: No observable effect

**Appendix C Response of biota to copper concentrations between 20-50 µg/L .  
\*denotes studies examining the ecological responses at specific mines or ARD  
affected regions.**

Type of Biota	Dissolved Cu (µg/L)	Observed effect	Type of study	Reference
Algae	40	Decrease in algal density*	Field - North Fork Iron Creek, Idaho	Genter and Lehman, 2000
	50	Decline in marine algae species	Field	Correa et al., 1999
Invertebrates	30	Benthos still impoverished*	Recovery - Ichi-kawa River, Japan	Watanabe et al., 2000
	42	100 % mortality	Caged snails in field (40days)	Reed-Judkins et al., 1998
	50	Decline in marine invertebrates	Field	Correa et al., 1999

**Appendix D Response of biota to copper concentrations between 50-150 µg/L.\* denotes studies examining the ecological responses at specific mines or ARD affected regions.**

Type of Biota	Dissolved Cu (µg/L)	Observed effect	Type of study	Reference
Algae	64	Loss of some species & change in dominance of periphyton taxa	Outdoor (12 day)	Soldo and Behra, 2000
	64	NOE on Chlorophyta green algae	Outdoor (12 day)	Soldo and Behra, 2000
	76	Shift from diatom to cyanobacteria dominant biofilms	Artificial stream	Barranguet et al., 2003
	80	Loss of some species*	Field – Norway	Lidstrom and Rorslett, 1991 cited in Gausch et al., 2002
	110	Reoccurrence of some previously lost taxa	Recovery in the field	David, 2003
	140	Decline in diatom biomass resulting in a bacterial dominated biomass	Mesocosm	Havens, 1994
Invertebrates	100	Reduced diversity and shift in dominance	Review	Reed and Gadd, 1990
Fish	120	Significant alterations to fish communities*	Field - Khan and Kshipra rivers, India	Ganasan and Hughes, 1998

NOE: No observable effect

**Appendix E Response of biota to copper concentrations >150 µg/L\* denotes studies examining the ecological responses at specific mines or ARD affected regions.**

<b>Type of Biota</b>	<b>Dissolved Cu (µg/L)</b>	<b>Observed effect</b>	<b>Type of study</b>	<b>Reference</b>
Algae	150	Min. algicide concentration for filamentous and planktonic (pH 7.2-7.4)	Algicide product info	Various
	250	Decline in marine algal species	Field	Correa et al., 1999
	318	Reduction in some periphyton, change community dominance,; dominance of unicellular green algae	Outdoor (12 day)	Soldo and Behra, 2000
Invertebrates	260	Complete loss of benthos	Field	Okada and Uesugi, 1958 cited in Watanabe et al., 2000
	>500	Complete loss of all benthos*	Field-Marcopper Mine, Phillipines	David, 2003
Fish	166	Decline in abundance of chum salmon fry*	Field – Howe Sound, Canada	Barry et al., 2000