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ALUMINIUM AND ACID RAIN: MITIGATING EFFECTS OF NaCl ON ALUMINIUM TOXICITY TO BROWN TROUT (SALMO TRUTTA FARIO) IN ACID WATER*

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The comparison of a fish stocking experiment in a Swiss mountain lake (Lake Laiozza) with results obtained in a South Norwegian lake (Lake Liervatn) revealed contradictory results as to the toxicity of the respective acid water. This, even though the pH, aluminium concentration, conductivity, and ionic composition of the two lakes proved to be almost identical. Lake Liervatn water was less toxic and had a substantially higher NaCl concentration. In order to answer the question whether NaCl could have a mitigating effect on pH-aluminium toxicity to fish, experiments were performed in the laboratory using "Synthetic Laiozza", a media made up from deionized water and salts added according to the concentrations found in Lake Laiozza. Synthetic Laiozza was then enriched with 0, 0.125, 0.25, 0.5, and 4.0 meq NaCl per liter media.

The addition of 0, 0.125, 0.25 and 0.5 meq NaCl/L had no significant effect on the survival time of the fish (all MT_{50}'s laying between 16 and 23 hours), whereas the addition of 4.0 meq NaCl/L resulted in longer survival of the fish i.e. $MT_{50} = 85$ hours. The analyses of plasma electrolytes on the other hand, revealed a progressive reduction in electrolyte loss with increasing ambient NaCl concentration.

KEY WORDS: Aluminium, toxicity, mitigation, ionoregulation, NaCl, brown trout, gills, electrothermical atomic absorption spectrometry (ETAAS).

INTRODUCTION

One and two year old brown trout (Salmo trutta fario) were stocked in the chronically acid Lake Laiozza, a poorly buffered mountain lake in the alps of southern Switzerland. This lake water (labile-Al = 41–52 μg Al/L, 0.5 mg Ca/L, 0.09 mg Na/L. Conductivity 7 μS/cm, and pH 5.37 $\pm$ 0.22) proved to be acutely toxic to both age classes ($MT_{50} = 37$ hours for one year old fish and 39 hours for the two year old fish respectively). All dying fish had elevated hematocrits (51–81 %) and extremely lowered plasma sodium and chloride concentrations i.e. 63–70 meq Cl/L and 93–109 meq Na/L respectively.[1] Skogheim and Rosseland,[2] on the other hand, reported only sublethal

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physiological stress in presmolt Salmo salar, a species more susceptible to pH-Al intoxication than Salmo trutta fario, exposed under almost identical conditions in a field experiment in Lake Liervatn, southern Norway. A comparison of Lake Liervatn water with the water of Lake Laiozza revealed a slightly higher Ca concentration and a substantially higher conductivity i.e. 60 μS/cm in Lake Liervatn. The higher conductivity was attributed to the "very high NaCl concentration in the water".[2] Further evidence as to the possibly mitigating effect of NcCl on aluminium toxicity to fish was found in Wright and Snekvik's survey on the chemistry and fish population status of over 700 lakes in southernmost Norway.[3] Of 84 lakes laying in a 10 km wide south-north transect, all having a pH equal or lower than pH 5.0, thirty-five are situated within 40 km of the coastline and have significantly higher NaCl concentrations than the ones situated in the mountains. The higher NaCl concentrations seem to mitigate pH-Al toxicity, as 63 % of these lakes maintain sparse or good Salmo trutta f. populations, whereas only 27 % of the lakes situated in the mountains i.e. with low NaCl concentrations are sparsely populated and the rest are barren.

MATERIALS AND METHODS

All experiments were performed with approx. 2-year-old brown trout (Salmo trutta fario) purchased at a commercially run fish hatchery in Andelfingen, Switzerland. Every experiment was carried out with ten fish and lasted 96 hours or until 50 % of the exposed fish had turned over (MT_{50}). The fish were not acclimatized to the experimental conditions with exception for temperature i.e. the fish were exposed to the testing water right after transport. The experimental setup used was a temperature-controlled ($10 \pm 1°$) recirculating system (436 liters) with 50 % media renewal every 24 hours. pH was kept constant by a titration unit coupled with a Metrohm 654 pH-meter. Sufficient airation was achieved by reintroducing the water by way of injectors. The exposure media "Synthetic Laiozza" was made up from deionized water adding the necessary ion according to the concentrations found in Lake Laiozza (Table 1).

As soon as a fish had overturned a blood sample was drawn by heart puncture.[4] The blood samples were analyzed for hematocrit and then centrifuged at 3000 rpm's for 10 minutes in order to separate the plasma. Na and K in the plasma were analyzed with the RA-1000 system (Technicon Instruments Corp., USA) using a glass sodium-sensitive electrode and a valinomycin membrane potassium-sensitive electrode.

Table 1 Major constituents measured in Lake Laiozza used for mixing the "Synthetic Laiozza" test media

Sodium	0.09 ± 0.01 mg Na/L	Labile aluminium	45 ± 18 μg Al/L
Potassium	0.20 ± 0.07 mg K/L	Conductivity	6.7 ± 0.8 μS/cm
Calcium	0.90 ± 0.31 mg Ca/L	Oxygen	9.5 ± 0.3 mg O_2/L
Magnesium	~0.06 mg Mg/L	Temperature	10.0 ± 1.0°C
Chloride	~0.11 mg Cl/L	pH	5.35 ± 0.05
Total aluminium	105 ± 9 μg Al/L		

Chloride was analyzed by coulorimetric titration on an Analyzer 929 (Corning Ltd., UK).

The aluminium concentration as well as the Na, K, and Ca concentrations in the media were controlled by frequent analysis of water samples using electrothermalatomic absorption spectrometry (ETAAS). Aluminium was speciated basically using the technique described by Barnes[5] and later modified by LaZerte[6] though no dialysis or cation exchange resin steps were carried out, but an extra MIBK (Methyl-Isobutyl-Ketone) extraction step introduced in turn.[7]

RESULTS

Acute toxicity

Addition of NaCl to the "Synthetic Laiozza" media significantly increased the MT_{50} values only when 4 meq NaCl/L were added. All other additions did not significantly increase survival time (Figure 1). In order to reproduce these results a retrial was started with additions of 0.5 and 4.0 meq NaCl/L respectively. Again a significantly higher MT_{50} value was recorded for the 4 meq NaCl/L addition experiment. The MT_{50} values of the retrials though were lower than the values reached in the first run (Table 2). This may be explained in part by the higher temperature difference between the rearing temperature and the experimental temperature in the retrials compared to the first run, and thus to a higher temperature acclimatization stress.

Plasma ion loss

In order to compare the ion losses of experiments with different duration i.e. MT_{50}'s

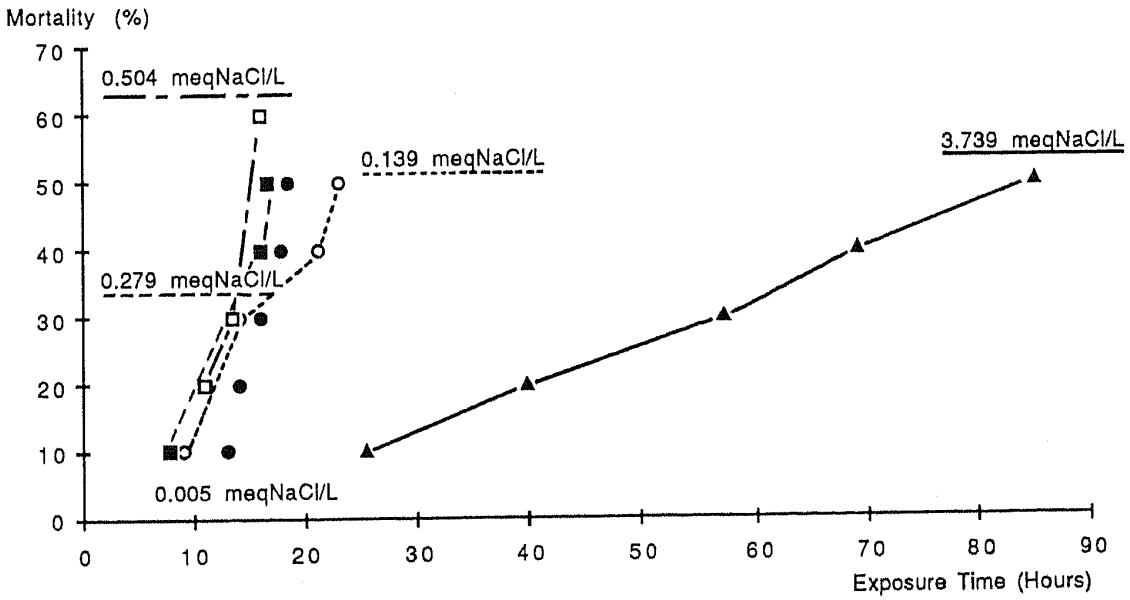

Figure 1 Cumulative mortality of brown trout exposed to 100 μg Al/L at pH 5.35$\pm$0.05 vs ambient NaCl concentrations.

the respective mean plasma ion concentrations had to be standardized. A "Mean Ion Loss" (MIL) per liter plasma and hour of exposure was calculated:

$$MIL = \frac{MNC - MEC}{MT_{50}}$$

Mean Normal Conc. (MNC): "Mean plasma ion concentration reported as normal for undisturbed and unexposed fish"

Mean Experimental Conc. (MEC): "Mean plasma ion concentration found in the respective exposures"

Manifestation Time 50% (MT_{50}): "Time until 50% of the exposed fish had lost their ability to keep an upright position"

The values used as MNC were control values taken from fish at the hatchery. These values compared well to values considered as normal in the literature.[8,9,10,11] A mean value of 160 ± 10 meq/L for Na and 130 ± 10 meq/L for Cl was therefore used for calculations. The addition of NaCl to the ambient water clearly reduces the net ion loss per hour of exposure (Table 2). The slightly higher ion losses encountered in the second experiment with 0.25 and 0.5 meq/L NaCl addition compared to the first experiment with 0 and 0.125 meq/L NaCl addition may be explained by small differences in sensitivity of the fish (Figure 2).

In order to compare the hematocrit values recorded in the respective NaCl additions a standardization with a normal hematocrit of $40 \pm 5\%$[12] had to be carried out in analogy to the MIL calculations, and thus an artificial parameter called Mean Hematocrit Increase (MHI) per hour of exposure was introduced (Table 2). As shown in the MIL calculations, the MHI increases more slowly with increasing ambient NaCl concentration, possibly reflecting a direct correlation of ion loss to the increase in hematocrit.

Table 2 50% mortality, calculated hematocrit increase (MIH), and mean ion loss (MIL) values vs ambient NaCl concentrations

*NaCl additions meq*L*	*0*	*0.125*	*0.25*	*0.5*	*4.0*
MT_{50} (hours)	18.5	23	16.5	16.0	85
MHI %/hour	0.973	0.652	1.030	0.813	0.165
Mean Na loss/hour	3.0	2.3	2.9	2.6	0.4
Mean Cl loss/hour	2.8	2.1	3.0	2.9	0.5
Repeated assays:					
Mt_{50} (hours)				11.75	30
MHI %/hour				0.851	0.667
Mean Na loss/hour				4.7	1.4
Mean Cl loss/hour				1.4	1.7

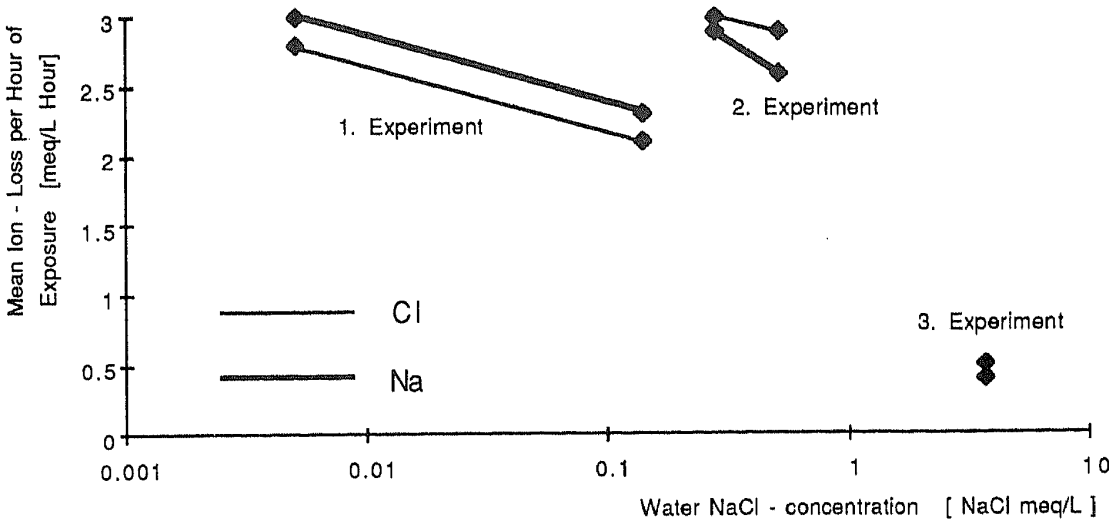

Figure 2 Mean ion loss (MIL) calculated vs ambient NaCl concentrations.

DISCUSSION

The mitigating effect of high NaCl-concentrations on pH-Al toxicity to fish cannot be explained by the reduction of the NaCl-concentration gradient between plasma and the ambient water, since this gradient of 160 ± 10 meq Na/L plasma to 0.5 and 4 meq Na/L water i.e. 130 ± 10 meq Cl/L to 0.5 and 4 meq Cl/L, respectively, practically remained unchanged. Nor did the analysis of the three major cations i.e. Na, K, and Ca throughout the duration of the experiments reveal any substantial variation in concentration such that an experimental bias had to be expected. The ambient Ca concentration did vary by a maximum of a factor 2, but higher calcium concentrations i.e. 0.029 meq Ca/L were recorded in low as well as high NaCl addition experiments without any significant influence on the survival times (Table 3).

The speciation of aluminium did not show any significant change in labile—Al concentration, and thus the exposure of the fish to the various Al-species can be considered as being almost identical in all experiments (Table 4). The labile—Al concentration in the first 4 meq NaCl/L addition experiment was significantly ($p < 0.05$) lower than in three of the four preceding experiments with lower NaCl

Table 3 NaCl additions vs values analysed in the water samples by ETAAS

NaCl additions meq/L		*0*	*0.125*	*0.25*	*0.5*	*4.00*
meq/L analysed	Na	0.005	0.139	0.279	0.504	3.739
	K	0.003	0.004	0.007	0.007	0.005
	Ca	0.016	0.029	0.016	0.022	0.018
Repeated assays: meq/L analysed	NA				0.496	3.913
	K				0.006	0.007
	Ca				0.016	0.029

Table 4 Aluminium speciation vs ambient NaCl concentrations. * denotes a significant difference ($p < 0.05$, Wilcoxon, Mann-Whitney 2-tailed U-test) to the corresponding values at lower ambient NaCl conc.

NaCl additions meq/L	*0*	*0.125*	*0.25*	*0.5*	*4.0*
Total Al μg/L	106 ± 2	103 ± 13	99 ± 6	108 ± 1	107 ± 12
>0.22 μm insoluble Al μg/L	36 ± 19	45 ± 6	36 ± 30	34 ± 22	62 ± 17 *
Labile Al μg/L	60 ± 18	48 ± 19	52 ± 22	67 ± 21	33 ± 7
Repeated assays:					
Total Al μg/L				111 ± 15	103 ± 12
>0.22 μm insoluble Al μg/L				12 ± 5	26 ± 23
Labile Al μg/L				81 ± 15	63 ± 23

addition. This result represents an experimental artefact as the water samples of this experiment were not speciated within the same time as the water samples of all the other experiments. That the aluminium speciation remains the same irrespective of the NaCl concentrations added can be seen when comparing the speciation of the 4 meq NaCl/L retrial with the speciation of the preceding experiments. These observations are corroborated by the findings of Leivestad *et al.*[13]

It is therefore possible to assume that the effects on survival time found during these experiments are solely due to the NaCl additions. As the ion efflux from the plasma into the ambient water is a function of the gill permeability, which in turn is controlled by the ambient Ca concentration and not by the NaCl concentration,[14] it can be assumed that the ion efflux rate remained unchanged in all experiments. The measured ion losses per hour reflect the net ion loss per hour i.e. (ion influx − ion efflux). Therefore, a reduction of net ion loss must reflect a change in the ion influx rate. Electrolyte uptake mechanisms e.g. Na-K ATP'ase have been shown to be inhibited in fish exposed to 27 μg Al/L at pH 5.8[15] and to 54 μg Al/L at pH 4.0 to 4.5.[16] A stimulation of Na and Cl uptake or a reduction of Na-K-ATP'ase inhibition by competition of Na or Cl with aluminium, may be two possible mechanisms by which high ambient NaCl-concentrations could mitigate pH-Al toxicity and lead to the reported reductions in ion loss per hour. The latter possibility was already mentioned by Leivestad *et al.*[13] who found lower inhibition of Na-K-ATP'ase when 0.3% sea water were added to experimental waters. Given the assumption that ambient Ca regulates only the permeability of the gills to water and electrolytes, and that ambient NaCl concentrations influence electrolyte uptake and/or enzyme inhibition by aluminium, it is likely that the ratio of NaCl to Ca concentrations in the ambient water, and not only the Ca-concentration, determines the degree of aluminium toxicity to fish.

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REFERENCES

1. D. R. Dietrich, PhD-thesis, 1988.
2. O. K. Skogheim and B. O. Rosseland, *Bull. Environ. Contam. Toxicol.* **37,** 258 (1986).
3. R. F. Wright and E. Snekvik, *Verh. Internat. Verein. Limnol.* **20,** 765 (1978).
4. J. Lehmann and F-J. Stürenberg, *Fisch und Umwelt* **8,** 77 (1980).
5. R. B. Barnes, *Chem. Geol.* **15,** 177 (1975).
6. B. D. LaZerte, *Can. J. Fish. Aquat. Sci.* **41,** 766 (1984).
7. D. R. Dietrich, in preparation (1988).
8. C. M. Neville, *Can. J. Fish. Aquat. Sci.* **42,** 2004 (1985).
9. M. G. Wheatley *et al.*, *Respiration Physiology* **55,** 155 (1984).
10. S. Thomas and G. M. Hughes, *Respiration Physiology* **49,** 371 (1982).
11. D. G. McDonald *et al.*, *J. exp. Biol.* **88,** 109 (1980).
12. J. Lehmann and F-J. Stürenberg, Gewässer und Abwässer (H. Kaltenmeier Söhne, Krefeld-Hüls, 1976), Vol. III, 7.
13. H. Leivestad *et al.*, *Annis. Soc. R. Zool. Belg.* **177/1,** 387 (1987).
14. P. G. McWilliams and W. T. W. Potts, *J. comp. Physiol.* **126,** 277 (1978).
15. H. Kjartansson, PhD-thesis (University of Bergen, Norway, 1984), 93 pp.
16. T. R. K. Dalziel, *Annls. Soc. R. Zool. Belg.* **177/1,** 421 (1987).