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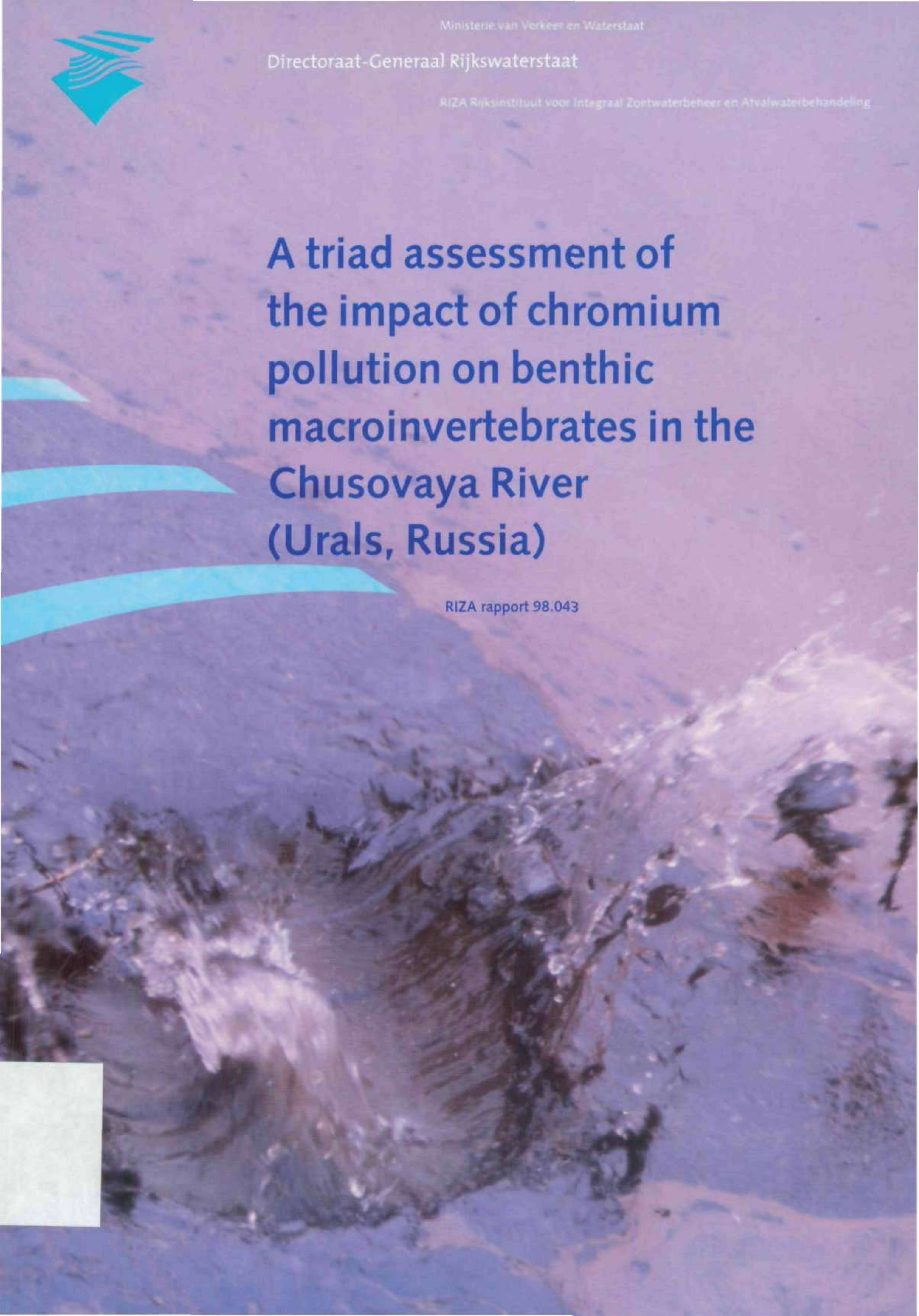

A triad assessment of the impact of chromium pollution on benthic macroinvertebrates in the Chusovaya River (Urals, Russia)

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A triad assessment of the impact of chromium pollution on benthic macroinvertebrates in the Chusovaya River (Urals, Russia)

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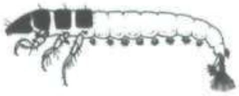
TABLE OF CONTENTS

TABLE OF CONTENTS (continued)

FOREWORD

The project described in this report took place within the framework of the cooperation between the Russian Research Institute for Complex Utilization and Protection of Water Resources (RosNIIVH) in Ekaterinburg and the Dutch National Institute for Inland Water Management and Wastewater Treatment (RIZA) in Lelystad. After the signing of a Memorandum of Understanding (MOU) in 1991 by official representatives of both Russia and the Netherlands, researchers from these two institutes came into contact with each other. A cooperation program followed, currently under the direction of two project managers, Mr. Timur Pavluk (RosNIIVH) and Mr. Abraham bij de Vaate (RIZA). I was able to make a contribution to the project RI-1811 "The distinction of different types of water pollution with the help of macroinvertebrates". The proposal to carry out the final research project of my masters degree studies at the University of Amsterdam within this program was approved the project managers and their institutes, RIZA and RosNIIVH.

The aim of this cooperation is to gather important information on the effects of different types of pollution on aquatic ecosystems. This information is valuable to water managers in both countries who are involved in the design and execution of biomonitoring programs and remediation of contaminated sites. As a supplementary research project, the Chusovaya River was selected to carry out a triad assessment of the impact of chromium contamination on the ecosystem. This river has been the subject of earlier studies both within the framework of the cooperation and by other researchers.



ABSTRACT

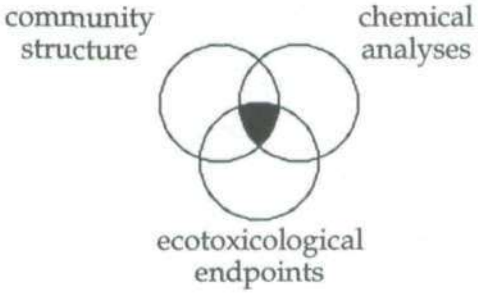
The impact of chromium (Cr) contamination on the benthic macroinvertebrate community of the Chusovaya River in the Ural Mountains of Russia was assessed using a triad approach. The triad consisted of chemical analysis of the contamination in various environmental compartments, examination of the benthic macroinvertebrate community structure, and analysis of ecotoxicological effects on the caddisfly *Hydropsyche pellucidula* (Trichoptera). Chemical analyses of water, sediments and detritus indicated that the main contaminant present was indeed Cr, and that the level of the Cr contamination near the point source, a severely polluted, dead tributary, was extremely high: downstream Cr concentrations were about 450 times higher in water and 25 times higher in sediments compared to a clean reference site upstream. The contamination at the mouth of the tributary was even more severe: 800 times more Cr in water and 50 times more Cr in sediments. Benthic macroinvertebrate community structure was studied by sampling using artificial substrates incubated in situ. Lower species richness was observed at the downstream site compared to the upstream site. Disturbances to the functional structure of this community were manifested in the absence of trophic groups. Larvae of *Hydropsyche pellucidula* collected from the polluted site on the river bioaccumulated large amounts of Cr and exhibited physical abnormalities. The incidence of tracheal gill damage was significantly higher than at a reference site on the nearby Reshotka River, as was the incidence of discolouration of the anal papillae of these animals. The application of a triad demonstrated that the observed extreme Cr contamination had an adverse affect on aquatic life in the Chusovaya River, both on community level (reduced diversity) and at the level of individuals (sublethal effects on surviving individuals).

1.0 INTRODUCTION

1.1 Triad assessments

Water or sediment quality assessments can involve a *triad* type of approach (Chapman, 1986; Van de Guchte, 1992), which is useful in studying river pollution problems (*e.g.* Canfield *et al.*, 1994; Den Besten *et al.*, 1995). The strength of this approach is the integration of knowledge attained through study of 1.) chemical analyses of the various environmental compartments 2.) benthic macroinvertebrate community structure and 3.) ecotoxicological endpoints (see Figure 1.1). The community structure angle of triad assessments provides an indication of the adverse effects on a functionally and taxonomically diverse community which plays a key role in the ecosystem. The extent of the effects of the contamination measured is thus indicated. The ecotoxicological angle acts as another link between the interaction of contamination and specific effects in selected key species, which may be in terms of bioaccumulation or other ecotoxicological endpoints such as mortality, growth, behaviour, or in the case of the present project, physical abnormalities. Adding this sensitive angle means that the impact on species level is also addressed, and achieves a more in depth investigation of the effects on the species which are still present, or in the case of laboratory tests, on cultured organisms which could potentially come in contact with the contamination in the field.

Figure 1.1 *The triad approach to evaluation of the condition of ecosystems involves study of three aspects: chemical analyses, community structure, and ecotoxicological endpoints.*



1.2 Chemical analysis

The nature of the contamination in the system under study must be investigated by means of chemical analysis. In fact, chemical analysis forms the basis of all assessments, regulation, and monitoring of aquatic ecosystems. It is the only way to identify the compounds responsible for degradation of a system and to determine at which levels they are present. In the case of trace metals, the speciation is generally very important for the solubility, mobility, binding character, bioavailability and toxic action of a metal. Environmental assessments of rivers are sometimes limited to the analysis of water samples. This gives a good indication of the extent of contamination of the water column. However, some contaminants are more concentrated in the beds of aquatic systems, and therefore, analysis of sediments may provide a more comprehensive picture of the distribution of the contamination. Closely associated with the sediments is decomposing plant material, or detritus. Because detritus is a very important energy source at the basis of the food chain, the concentration of the contamination in it may be worth determining. Organic matter may act as a sink for some types of contamination, such as hydrophobic compounds which quickly leave the water phase to adsorb to organic matter (*e.g.* Kukkonen & Oikari, 1991), or cations which are attracted to its many available negatively charged binding sites (Stumm & Morgan, 1981).

The existence of river pollution may go undetected by standard chemical analyses due to the fact that chemical sampling is not continuous, and represents only discrete moments in time and space. Also, concentrations may be below detection limits, although some chemicals remain very toxic even at low concentrations. And there is a limit to the number of compounds that can be screened in standard analysis series. Effects not detected by chemical sampling are often effectively signalled by changes in benthic community structure (Johnson *et al.*, 1993).

1.3 Benthic macroinvertebrate community structure

Biological systems are better indicators of bioavailability and combination toxicity of pollution than chemical analyses. Furthermore, organisms integrate the environmental condition over time. This is not possible with discrete chemical sampling alone.

Benthic macroinvertebrate community structure plays and important role in modern water quality assessment programs in a growing number of countries (Chapman, 1994). Insight into the condition of aquatic ecosystems attained through studies of benthic community structure is valuable because of the extra information this provides about the condition of the water and aquatic sediments. Because the habitat of benthic macroinvertebrates is situated at the interface of sediment and water compartments, these organisms can facilitate the transfer of sediment-associated contaminants to the water column. These bottom-dwellers are in the unique position of being directly exposed to both water and sediment contamination. This community represents numerous phyla and trophic levels and is relatively easy and inexpensive to sample. It's central position in the trophic structure of an aquatic ecosystem makes it suitable to use as an indicator for the condition of the ecosystem on the whole (Pavluk, 1997).

There is a variety of approaches to bioassessment among which traditional methods, such as those based on the saprobic system (Pantle & Buck, 1955), which may be used to indicate organic pollution. Diversity indices are used to characterize the species richness, evenness and abundance in a community, and assume that all healthy ecosystems have a naturally high level of diversity. The Shannon index (Shannon & Weaver, 1949) is an example of a commonly used diversity index. Biotic indices, such as the Trent Biotic index (Woodiwiss, 1980) or the Belgian Biotic index (De Pauw & Vanhooren, 1983), sharpen the power of diversity indices by adding an indicator species component, resulting in a single score for the level of disturbance to the ecosystem.

In the case of aquatic invertebrates, attempts to improve upon some shortcomings of the above-mentioned approaches to biological assessment have lead to the development of what is known as the functional approach. As described by Cummins (1988): "the functional view permits clustering of genetically and taxonomically diverse entities into groups, or guilds, which share fundamental properties - such as invertebrates having the same morphological-behavioural mechanisms of food acquisition". Taxonomy is less important than the function fulfilled by the members of the community, making its application not limited to narrow geographical zones. Based on this concept, a new index has been developed called the Index of Trophic Completeness, ITC (Pavluk, 1997). On the basis of defined trophic criteria, a total of 12 trophic groups were identified which should be present in any undisturbed aquatic ecosystem. The results can be presented using a pie graph, in which trophic groups not represented in the benthic macroinvertebrate community of the given ecosystem are indicated by a black wedge in the pie.

1.4 Ecotoxicological endpoints

While a study of the community can tell us if the ecosystem is in tact or not, studying ecotoxicological endpoints can indicate to what extent the members of the ecosystem are adversely affected by any contamination present. Ecotoxicological endpoints are sensitive measures of the effects on selected species and allow an assessment to zoom in on more subtle effects in surviving species. Ecotoxicological testing may take many forms. Organisms from laboratory cultures may be exposed to water or sediments collected from field sites (*e.g.* standard toxicity tests using such organisms as the water flea, *Daphnia magna*). Another approach is to collect organisms directly from the field and examine them for effects such as morphological abnormalities (*e.g.* in chironomids, Warwick, 1985). It is also possible to relocate healthy organisms to a field test site where their progress is followed *in situ* (*e.g.* Stuijfzand *et al.*, 1998; Ivorra *et al.*, submitted).

In addition to chemical analysis of the physical environment, the build-up of pollutants in the organisms themselves can be measured. Bioaccumulation may be considered to be an ecotoxicological endpoint and is useful as an indicator of bioavailability of contaminants and the potential risks associated with bioavailability. Bioaccumulation, which can contribute to disappearance of sensitive species and sometimes important trophic links (Pavluk, 1996) is especially important in rivers supporting fishing industries, as these organisms form the main component of the diet of benthic-feeding fish such as trout (*e.g.* Farag *et al.*, 1994). Emerging aquatic insects may even facilitate the transfer of metals which contaminate aquatic systems to land (Currie *et al.*, 1997) as they are preyed upon by various terrestrial insectivores.

Adverse effects on one member of the community may also tip delicate competition balances within the community which may lead to alterations in the community structure and niche relations (Dudley *et al.*, 1990; Petersen, 1986). It is plausible that even subtle influences on behaviour for example, could affect the intricate interactions of the aquatic ecosystem. For example, caddisflies have been shown to exhibit behavioural reponses to acute exposure to metals in low to intermediate concentration ranges (Vuori, 1994; Van der Geest *et al.*, 1997), which may affect balances within this taxa.

The exotoxicological angle of a triad assessment provides information additional to the data on which elements of the community are absent or present; the physiological stress of contamination can also be characterized.

1.5 A triad assessment of the impact of chromium on the Chusovaya River

The work presented in this report was carried out as a part of a larger program to study the effects of different types of pollution with the help of macroinvertebrates in three rivers with point sources of different types of pollution in the Ural Mountains region of Russia. One of the rivers under study, the Chusovaya, was selected for additional research described in this report. The Chusovaya River has its source in the southern Urals, flows northwestward where it empties into the Kama River, the major tributary of the Volga, which in turn flows into the Caspian Sea. The Urals region has a long history of metallurgical industry, and many factories and plants are still operating with suboptimal environmental standards.

Chromium (Cr) is the main contaminant in the Chusovaya River near the municipality of Pervouralsk. In this case, the source is a chromium salt processing plant, although Cr contamination commonly arises as a result of a variety of industries such as leather tanning, paint production, wood-preserving, textile, and graphics (Slooff *et al.*, 1989). Cr contamination levels from this source have been consistently high, exceeding the Russian norm for total Cr by well over 1000 times on average (Pashkevich & Pavluk, 1996). No pesticides are used in this area, and there are no industries producing organic micropollutants, and therefore these types of compounds have not been detected in the river water, except at very low concentrations (Pashkevich & Pavluk, 1996). Elevated levels of radioactive pollution have also not been detected in the Chusovaya at Pervouralsk (Stepanov *et al.*, 1992) despite the proximity of the river's source to severely radioactively-contaminated sites (*e.g.* Mayak in Chelyabinsk Oblast).

The speciation of Cr is very significant for its biological action. The two most stable oxidation states of Cr are the trivalent (poorly soluble), and hexavalent (very soluble and therefore mobile). There are seven other possible oxidation states of Cr although these are either too rare or too unstable to be found in natural aquatic systems (Slooff *et al.*, 1989; Pawlisz *et al.*, 1997). Cr(III) is an essential element for animal and plant metabolism and thus is not toxic at natural concentrations, while toxic effects are generally attributed to Cr(VI) (Janus *et al.*, 1989). This has to do with the ease with which Cr(VI) passes membranes. Once inside the cell, it is reduced to Cr(III), and may exert a variety of effects including genotoxic and carcinogenic (IARC, 1980; Itoh & Shimada, 1997). Cr(VI) is able to induce teratogenic effects by exposure via drinking water in a mammalian system (Kanojia *et al.*, 1997), while both Cr(III) and Cr(VI) have been linked to teratogenic effects after parenteral administration to pregnant hamsters and mice (WHO, 1988). The chronic and acute toxic effects of Cr(III) and Cr(VI) in both freshwater and marine environments have been extensively reviewed in Pawlisz *et al.* (1997).

In general, surface waters which are well-circulated and aerated are oxidizing. The redox state of Cr depends on a variety of factors, including redox potential (Eh), pH, presence of oxidating and reducing compounds, the redox reaction kinetics of Cr compounds, the formation of Cr(III) complexes and insoluble Cr salts. In aerated surface waters, thermodynamic calculations predict that Cr will exist primarily in the hexavalent form (Pawlisz *et al.*, 1997; Cranston, 1983; Slooff *et al.*, 1989). Hexavalent Cr dominates in surface waters, especially in well-aerated streams and small rivers, while the trivalent form is found in more reducing conditions, such as anaerobic sediments and wetlands (Pawlisz *et al.*, 1997). Cr(VI) is present in surface waters as a negatively charged hydrated oxo-compound, which does not tend to complex with inorganic or organic ligands. Cr(III) on the other hand, is a cation which can form hydroxides which can react with many possible ligands (*e.g.* sulfates, phosphates, phenols, caboxylic acids, ammonia) which are normally very insoluble.

In a classic study of the Eh-pH relationships in natural waters, Baas Becking *et al.* (1960) showed that most aerated surface waters are clustered in a line below and parallel to the upper boundary of water stability (the "irreversible oxygen potential"). In this range of an Eh-pH diagram for Cr (see Figure 4.1) the dominant species is Cr(VI). The results of earlier research on the Chusovaya River (Pashkevich & Pavluk, 1996; Stepanov *et al.*, 1992) indicate that the hexavalent form of Cr makes up more than half of the total Cr in the water of this river.

A triad assessment in the Chusovaya River, with its point source of Cr pollution, gives insight into the pathways that Cr (potentially) takes to exert its effects on the ecosystem and also the types of toxic effects that Cr can have on members of the benthic macroinvertebrate community. This very clear point source of Cr provides an excellent *in situ* opportunity to study the fate, movement and toxicity of Cr in the natural environment.

By measuring metals, particularly Cr, in water, sediment and detritus from sites upstream and downstream from the point of entry of the Cr pollution, it is possible to describe the distribution of the contamination over the environmental compartments. By sampling the benthic macroinvertebrate community and by applying indices to the species data, an estimation of the level of disturbance to the ecosystem can be made. And lastly, toxic effects on species level can be identified by focussing on the caddisfly *Hydropsyche pellucidula* (Trichoptera: Hydropsychidae), a key member of the benthic community (Paine, 1966; Higler, 1980; Hynes, 1970).

Other advantages of benthic macroinvertebrates in assessment studies include their sedentary nature which restricts them to more compact foraging areas than for example, fish, and lifespans of a suitable length for detecting effects of pollutants. This made it effective to collect caddisfly larvae from natural substrates in the field to analyse for metal bioaccumulation and examine for presence of physical abnormalities. Vuori (1992, 1994, 1996) has described darkening of anal papillae (the posterior ion exchange organs in the larvae of hydropsychids) and damage to the tracheal gills following metal exposure. These endpoints were examined as part of the ecotoxicological angle of the triad assessment.

2.0 MATERIALS AND METHODS

Over a four month period (June to September 1997), the benthic macroinvertebrate community and various other biotic and abiotic parameters were observed and measured in the Chusovaya River at sites upstream and downstream from a point source of Cr.

2.1 River study sites

Study sites were chosen on the Chusovaya River (Volga Basin) based on data available from the Urals Hydrometeorological Board, Ekaterinburg (unpublished data) and previous water quality studies in this river (Pashkevitch & Pavluk, 1996; Stepanov *et al.*, 1992). The Chusovaya has its source in the Chelyabinsk Oblast, flows northwestward through the Sverdlovsk Oblast into the Perm Oblast where it empties into the Kama River. A clean upstream reference site (56°48′ N, 60°00′ E) and a polluted site (56°52′ N, 59°55 E) downstream from the point source were selected in the Sverdlovsk Oblast, northeast of the city of Ekaterinburg (Figure 2.1). The downstream site, located near the city of Pervouralsk, was approximately 200 m downstream from the confluence with the Pakhotka River. At the mouth of this tributary, sediment sampling and screening for presence of surviving macroinvertebrates was performed. The upstream site, near the town of Revda, is approximately 14 km upstream of the confluence of the tributary and the Chusovaya. One other reference site was selected near the town of Reshyoti on the Reshotka River (in the same catchment and approximately 15 km northeast of the upstream site on the Chusovaya River) for the purpose of comparing bioaccumulation and sublethal effects on the caddisfly, *Hydropsyche pellucidula* (see section 2.6).

2.2 Water sampling and analysis

When visiting the sites for the first time (June 1997), and on the three following visits (July, August and September) to collect benthic macroinvertebrates, water samples were taken for analysis of metals. Water samples for metal analysis were collected in both glass (3 x 10 ml) and polyethylene (500 ml) bottles with screw caps leaving no head space, acidified to $pH<2$ with HNO_3 (analytical grade), and stored cool and in the dark. In addition, water samples were collected in glass bottles for analysis of the following: pH, O_2, Pt-Co colour scale value, Na^+, K^+, Ca^{2+}, Mg^{2+}, Fe^{2+}, Fe_{total}, HCO_3^-, SO_4^{2-}, Cl^-, NH_4^+, NO_2^-, NO_3^- N_{total}, P_{total}, P_i, suspended matter, BOD (5 or 7 d method), COD (Cr_2O_7 method). Water samples were also taken and analyzed for metals using the ICP-AES technique (analyses performed by OMEGAM Ltd. on samples from first sampling month) and the graphite furnace AAS (University of Amsterdam, the Netherlands) was also employed for the samples which were taken in the following months. A series of standard calibration solutions were made from $CrCl_3$ (Titrisol) in reagent grade water and HNO_3 (Baker pro analysis) according to Van Zwieten (1997). A solution of 5 g/l $Mg(NO_3)_2$ was used as the matrix modifier. Other water sample analyses were carried out by the Analytical Chemistry Department of RosNIIVH.

Temperature, conductivity and current velocity were measured on visits to the sites. Current velocity was measured using an A. Ott (Kempen) Type 12.400 velocity meter. At 1 m intervals across the width of the river, the current velocity was measured. At depths greater than 1 m, the velocity was measured at 0.2 × depth and 0.6 × depth. Profiles of the rivers were made and the data were extrapolated for the entire area for each river. Using this data, it was possible to calculate discharge (m^3/s), an important parameter for macroinvertebrates inhabiting riverbeds.

An indication of the redox state of the system could be calculated using the data on oxygen concentrations and pH. Using the Nernst equation, and assuming that oxygen was one of the major oxidants in this aerobic system, the Eh (redox potential) was calculated for each sampling date.

a)

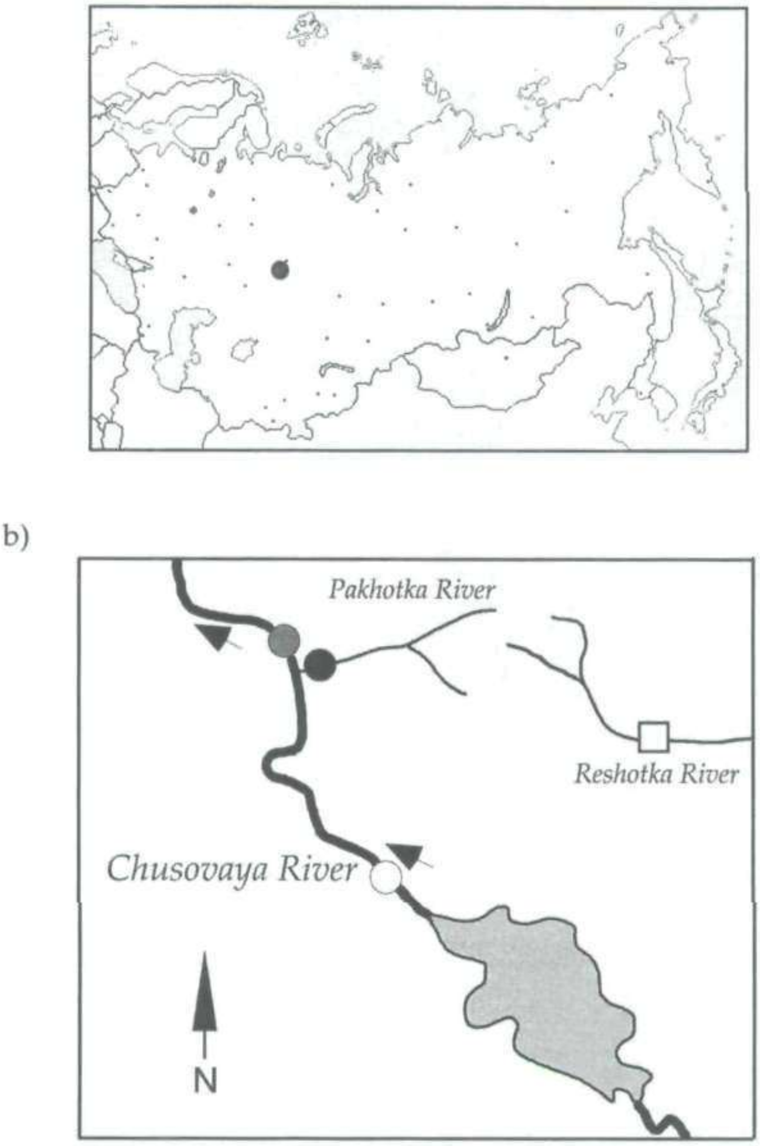


Figure 2.1 (a) *Map of CIS indicating approximate geographical location (gray circle) of study area shown in (b) in detail.* (b) *Schematic drawing of sites visited on the rivers Chusovaya (clean until point source), Pakhotka (polluted), and Reshotka (clean). Chusovaya upstream site (white circle); Chusovaya downstream site (gray circle); Pakhotka site (black circle); Reshotka site (white square). Scale: 1 cm = 2.5 km.*

2.3 Sediment sampling and analysis

Sediment sampling was carried out during the last visits to the sites (September). Nine sediment samples were taken from the reference site, six from the downstream site and three from the mouth of the polluted tributary. Each sample was mixed, and split into three subsamples for analysis of total metal content, bioavailable metal content, and finally grain size, organic content and total carbonate concentration (% w/w). The sediment samples for metal analysis were collected in 100 ml plastic bottles with screwcaps, leaving no headspace, as for the water samples. To samples for total metal content, HNO_3 (analytical grade) was added until pH<2, and samples were stored cool (±4 °C). Samples for the bioavailable metal fraction analysis were stored cool and later frozen without conservation. Samples for grain size, organic content and calcium analyses were collected in plastic ziplock bags, frozen, and also transported in a frozen state to the Netherlands to prevent any degradation of the organic material.

2.3.1. Total metals in sediment

The extraction method used for the analysis of total metals in sediments agrees with the NVN 5570 standard protocol (1993). Samples for total metal analysis were first freeze-dried and homogenized with an agate mortar and pestle, but not ground. At the same time the samples for metal analysis were being weighed, extra subsamples were taken to be able to measure moisture content and loss-on-ignition (NEN 5754, 1992) for each sample. Per sample, 0.5 ± 0.1 g (to the nearest 0.0001 g) of dry sediment was weighed out *in duplo* into 120 ml teflon vessels for the extraction of metals.

To each vessel, 12.0 ml 37% HCl (Merck, pro analysis) and 4.0 ml 65% HNO_3 (Merck, pro analysis) were added and left to ventilate overnight. Blanks and internal standards (Amsterdam canal sediments, prepared by University of Amsterdam, Physical Geography and Soil Sciences Department) were also used and analysis of seven metals gave results at least within 10% of the standard values. The teflon vessels were placed in a microwave oven (CEM MDS-81D) for a 110 minute program in which the power was increased stepwise. After this procedure, the sample solutions were quantitatively transferred to 100 ml volumetric flasks and reagent grade water was used to bring the volume to 100 ml. Concentrations of Cd, Cr, Cu, Mn, Ni, Pb, and Zn in these solutions were measured using a Perkin-Elmer 5000 flame AAS (impact bead, acetyleen-air, 5 seconds, measurements *in duplo*).

Absolute dry weights in each teflon vessel were calculated using the ratio of the dry weight after freeze-drying and the measured water loss after 105 °C for corresponding samples in the moisture content analysis. Sediment metal concentrations were then expressed in µg/g dry weight sediment. (The convention of using units of mg/kg was avoided in this case for the sake of simpler comparisons between different environmental compartments which are expressed as µg/g).

The differences in metal contents between sediments from the different sites were compared using an ANOVA test where conditions for it were satisfied, and otherwise a non-parametrical (Kruskal-Wallis) test.

2.3.2 Bioavailable metals in sediment

Bioavailable metals (exchangable fraction) were extracted using a $CaCl_2$ extraction method, originally developed for estimation of bioavailablity of metals to plants, but now considered to be a good method to assess metal bioavailability to benthic invertebrates as well (K. van Gestel, personal communication). The method used was based on methods for extraction of soils described by Houba *et al.* (1994) and Verweij (1997).

Sediments were kept at 4 °C or lower until analysis. The wet sediment was first placed in an oven at 40 °C until dry (± 24 h), then homogenized (but not ground). Each sample was then analysed *in duplo* by weighing 5 ± 0.1 g (to the nearest 0.0001 g) dried sediment into 100 ml acid-rinsed polyethylene bottles. At the same time, per sample, two 2 ± 0.1 g subsamples were taken for moisture content and organic material determination. To each container, 50 ml of a solution of 0.01 M $CaCl_2$ ($CaCl_2 \cdot 2H_20$ "Baker" reagent) in reagent grade water was added by weight. The lids of the containers were secured and the samples were mixed using an automatic shaker at 200 rpm for 2 hours. After centrifugation of the suspensions in the polyethylene bottles (10 min. at 2000 rpm) the pH of the extracts was determined. The resulting solution was decanted, transferred to plastic, acid-rinsed test tubes and centrifuged (15 min., 3000 rpm). Known volumes of the supernatants were collected and acidified with HNO_3 to bring the solutions to the same pH as the standard calibration series.

The concentration of $CaCl_2$ (0.01 M) was low enough not to cause interference to the AA measurements. The Cr standard calibration solutions already contained chloride ions and no interference of Ca was evident in the sample blanks. The metals Cr, Cu and Fe were analyzed in the extracts by graphite furnace atomic absorption as for water and detritus analyses. The absolute dry weights of the wet sediment samples were calculated from the moisture content analysis. Organic content was again calculated from the weight loss after 16 h at 375°C. Metal concentrations were then expressed as μg/g dry weight sediment. Each sediment sample was analysed *in duplo* for metals.

2.3.3 Organic content, grain size, total carbonate analysis

Other parameters in sediment samples were measured by OMEGAM Ltd. in Amsterdam: grain size, total carbonate and organic content. In the grain size analysis, the breakdown of the different fractions (<2, <10, <16, <63, and <210 μm) were given as percentages (w/w), of which the <2 and <16 μm fractions were determined by sedigraph. Total carbonate was determined according to the NEN 5757 protocol (1991) and expressed as g/kg dry weight. Dry rest and loss-on-ignition were calculated as percentages of the dry weight.

2.4 Detritus sampling and analysis

Detritus samples were taken at each site on the last sampling visit by gathering detrital material from the substrate near the riverbank. Detrital particles were separated from sediment and other non-detrital particles by adding river water to a bucket containing the materials, and creating turbulence with a plastic scoop. The suspension was left to settle for about one minute, after which the top part of the suspension was decanted into another bucket. The remaining heavier particles on the bottom of the first bucket used were discarded, and the process was repeated 3 times, each time taking the top suspension, and discarding what had settled to the bottom within the minute waiting time. The suspension of plant material which remained was allowed to precipitate overnight and was then collected. The plant material was dried at 70 °C until moisture had evaporated. It was then stored cool (±4 °C) in the dark until transport to the Netherlands was possible. Only the <850 μm fraction of the detritus was used, as these detrital particles are directly available as food for a large portion of the benthic macroinvertebrate community.

Following grinding and freeze-drying of detritus samples, subsamples of 2.5 ± 0.3 mg were weighed into 3 ml teflon vessels to the nearest 0.01 mg. A freeze-dried standard reference material (NIST 2704 Buffalo River sediment) and blanks were also run through the same procedure as the detritus samples. To all teflon vessels, 50 μl HNO_3 (Baker Ultrex) was added. The samples were then placed in a microwave (CEM MDS-2000) for a program of one hour to accelerate the acid extraction. After cooling, 2 ml of Aquadest was added to dilute the remaining acidic extract. Cr in the resulting solutions first had to be diluted in most cases before being measured using the graphite furnace AAS (Perkin Elmer 5100PC) as described for the analysis of water and bioavailable metal in sediment. The standard reference material was also tested for recovery of metals other than Cr, (specifically, Cu and Fe) to check the agreement of these values with certified values, and thereby to gain insight into the efficiency of the extraction.

2.5 Benthic macroinvertebrate community sampling and analysis

Macroinvertebrates were collected from Dutch Artificial Substrate Samplers following three colonization periods of approximately 4 weeks (34 ± 10d). At both the upstream and downstream sites, three samplers consisting of glass and/or ceramic marbles were installed. No samplers were placed in the tributary as this has been found to be incapable of supporting life (Pavluk, personal communication). This was confirmed during the sampling period: no animals were found in samples of the riverbed taken with a circular shovel. The contents of each sampler were collected by emptying the metal baskets onto a wide-meshed tray to catch the marbles, while collecting the organisms associated with them in a sieve at the bottom of a funnel under the meshed tray. Any organisms clinging to the metal basket itself were always excluded from the sample. The macroinvertebrates collected from each sampler were stored separately, preserved in alcohol and refrigerated until determination. Samplers were thoroughly cleaned with a brush, filled with cleaned marbles and returned to the river for the next colonization period.

Determination was conducted by consulting keys from both Russian and European sources. When a discrepency occurred between two keys, the Russian source was normally considered to be more accurate, being designed for species of Russian water bodies. After determination, the total wet weight of the individuals of each species was recorded to the nearest 0.005 g.

The species lists from each site were analyzed by applying Pavluk's ITC method (Pavluk, 1997). The ITC method uses a computer program (see Pavluk, 1997) developed for the calculation of the number of trophic groups present in benthic macroinvertebrate species lists, which is a measure of the condition of the ecosystem. A large number of organisms have been described in the literature and these have been assigned to a trophic group based on the principles of the ITC method. In addition, for purposes of comparison, the species data were tested using two other diversity indices, the Shannon index (Shannon & Weaver, 1949), and the Margalef index (Clifford & Stephenson, 1975). The Shannon index puts more weight on each species present being represented by many members (evenness) and is calculated with the formula:

$$H' = \sum \frac{Ni}{N} \log_2 \frac{Ni}{N}$$

where N_i is the number of individuals of species i, and N is the total number of individuals in the community. The Margalef index is calculated with the following formula:

$$H = \frac{(S-1)}{ln\,N}$$

where S is the number of species, and N is the total number of individuals in the community. It is a measure of species diversity in which the number of species (and not so much the numbers in which these species are present) is the most significant factor.

The results of evaluations using these various methods were compared.

2.6 Physical abnormalities and bioaccumulation in *Hydropsyche pellucidula*

Specimens of *Hydropsyche sp.* were collected by hand from natural substrates at the downstream site on the Chusovaya River and transported to the lab in river water. *H. pellucidula* were separated from other *Hydropsyche* species and used for further analyses. As the reference location on the Chusovaya had a rather low current velocity, it was not a prime habitat for the net-spinning caddisfly larvae, and thus this species was not abundant. A suitable reference site was found on the Reshotka River, within 15 km of the Chusovaya upstream reference location. The Reshotka is a clean river which serves as a canal for drinking water, and the existing chemical monitoring data shows a lack of pollution in past years (Gosudarstvennii komitet SSSR po gydrometeoroltii uralkoe territorialnoe uravlenia op gydrometeorologii, 1991). The water chemistry of this river is very similar to the Chusovaya at the reference site except for the slightly elevated total Cr levels (see Results 3.1). There, *H. pellucidula* was found present and were collected in the same way as at the downstream site. Reshotka river water samples were taken for general water chemistry and metals analysis.

After clearing of the guts while in river water (min. 24 h), *H. pellucidula* specimens (fifth instars) were examined for the presence of abnormalities in the tracheal gills and anal papillae as described by Vuori (1992, 1994, 1996). Vuori reported gill damage and discolouration of the normally clear or white-coloured anal papillae in *Hydropsyche* sp.

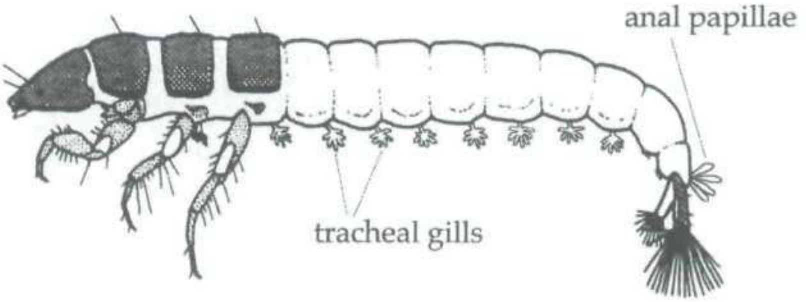
(including *H. pellucidula*) exposed to metals. These effects were scored in the collected animals (20 from reference site, 14 from downstream site).

The tracheal gills of *Hydropsyche* larvae are located on the ventral side of the abdomen, each gill consisting of a stalk with tufted branches (see Figure 2.2). Damage to the tracheal gills was easily identified by the darkening and reduction of tips of gill branches. A scale was defined to quantify the degree of damage: 0 = no damage; 1 = marginal damage (≤ 5 tips black); 2 = minor damage (>5 black); 3 = moderate (>25 black); 4 = extreme (all black). Extreme cases of gill damage were very rare. Gill damage was averaged within a site by averaging the scores of the individuals.

Anal papillae were examined and discolouration (darkening) was noted for each of the four papillae per individual. The percentage of individuals showing darkening in one or more of the papillae was compared between the two sites. From the reference site, the same larvae were tested as were examined for physical abnormalities, and from the downstream site, an extra five fourth instar larvae were included in the bioaccumulation analysis as there was no significant difference between in the data sets for fourth and fifth instar accumulation.

Following examination for physical abnormalities, the larvae were rinsed for 10 min. in acid (pH < 2) to remove metals bound to the exoskeleton since these are considered biologically inactive, and not important toxicologically. Larvae were placed in individual 2 ml round-bottomed eppendorfs, frozen and later dried at 70 °C. After transport to the Netherlands, they were further freeze-dried and weighed to the nearest 0.001 mg. A standard shrimp powder (IAEA MA-A-3/tm) was used for the standards, and four blanks were run. Extracts of the larvae were made by adding 200 µl HNO_3 (Baker Ultrex) to each eppendorf, which were contained in a 101 °C heating unit. After 4 hours, 100 µl HNO_3 (Baker Ultrex) was added, followed by 200 µl H_2O_2. When this had dried, 2 ml of reagent grade water was added. This solution was then measured for Cr and Cu using graphite furnace or flame AAS (Perkin Elmer 5100 PC).

Figure 2.2 *Diagram of a* Hydropsyche sp. *larva showing tracheal gills on the ventral side of the abdomen and four anal papillae at the posterior end. (Actual length, up to ± 2 cm)*



3.0 RESULTS

3.1 Metal concentrations and other water chemistry parameters

The analysis of metal concentrations in water revealed that the main pollutant in the Chusovaya River at Pervouralsk is clearly Cr (Figure 3.1). The average total Cr concentration over the sampling period at the downstream site was 922 ± 102 μg/l compared to 2 ± 1 μg/l at the upstream site. The polluted tributary was the most contaminated with Cr (6120 ± 147 μg/l). Cu and Ni concentrations were slightly higher at the downstream site compared to the upstream site (3 and 2.4 times respectively) although these levels are still low. The Reshotka River, where *H. pellucidula* were collected, showed a slightly elevated level of Cr (34.1 ± 9.7 μg/l) compared to the upstream site on the Chusovaya River (Figure 3.2).

The many parameters measured made it possible to gain a broad overview of the similarities and differences among the study sites (Appendix I). All waters were slightly alkaline and carbonate concentrations, important for the buffering capacity of the system, were similar at upstream and downstream sites on the Chusovaya, although somewhat higher in the water samples from the tributary. None of the waters in this study were nutrient rich, considering the low nitrogen and phosporous levels at all sites. Pt-Co colour unit values averaged 92 downstream and 40 upstream, while in the tributary, the value was considerably higher: 403. The amount of particulate matter detected in water samples showed considerable variation at both the upstream site, 9.4 (1.7-26) mg/l and the downstream site, 7.3 (2.1-13.7) mg/l.

Conductivity was lower at the upstream site (average 0.261 ± 0.0196 μS/cm) than at the downstream site (average 0.423 ± 0.0181 μS/cm), which was in turn four times lower than the conductivity in the tributary. The current velocity was somewhat higher at the downstream site (average 0.45 m/s) than at the upstream site, where it was normally 0.1 m/s. The flow regime was greatly changed during a one week period in August when discharge increased from about 0.2 m^3/s to as high as 49.6 m^3/s (Urals Hydrometeorological Board, unpublished data). This is because the discharge at the upstream site is under the influence of a reservoir dam. Sulfates were also determined in water samples, and although somewhat variable in the course of time, the concentrations downstream were nearly double the upstream concentrations due to the input from the tributary, where SO_4^{2-} concentrations reached 495.3 mg/l (five times higher than downstream concentrations).

The water chemistry analyses also show that the water quality of the Reshotka River where *H. pellucidula* were sampled as a reference agreed relatively well with the upstream Chusovaya site (see Appendix I). The Pt-Co colour value was more similar to the downstream site however (73).

From the Eh calculations, it appears that the redox potential values for the upstream and downstream sites on the Chusovaya as well as the Reshotka reference sites were very similar and that at all sites oxidizing conditions prevail (see Appendix I). Fe(II) was not detected in any of the water samples. These sites are characterized by very high oxygen levels.

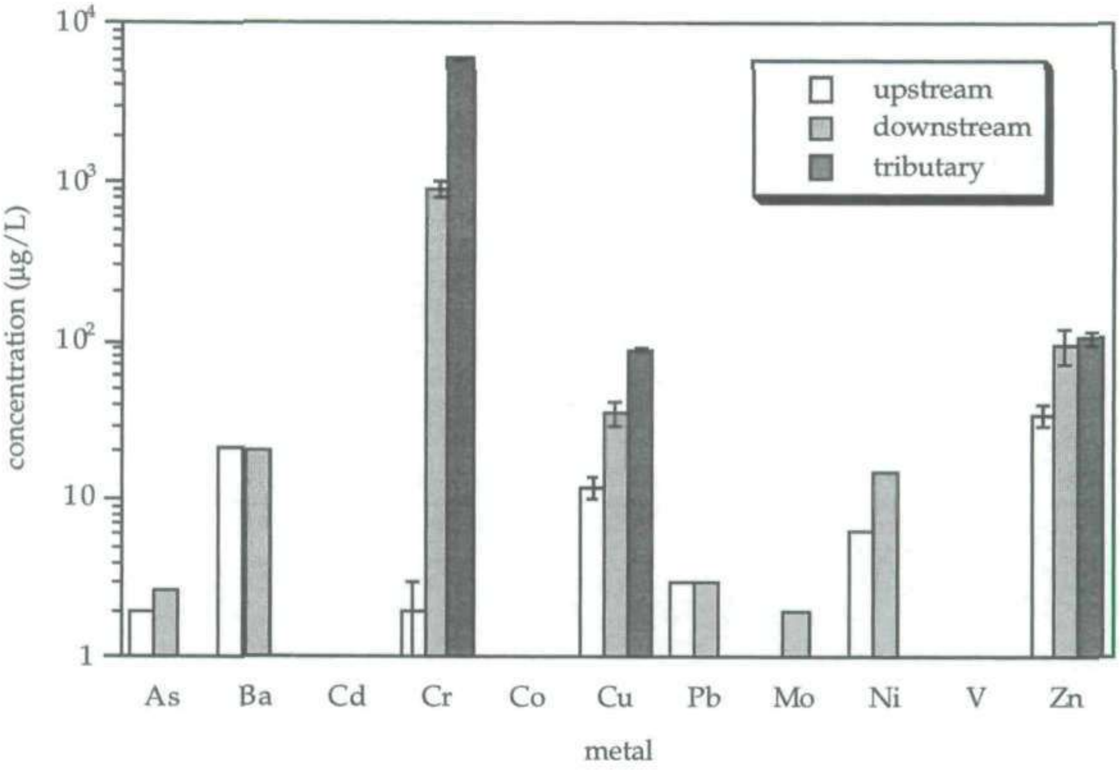


Figure 3.1 *Metal concentrations (µg/l) in water from Chusovaya River upstream and downstream sites and the Cr-contaminated tributary, the Pakhotka River. Data for Cr, Cu, Cd, and Zn is averaged for the sampling period (June to September 1997) and standard error bars indicate the fluctuations in the time. Other metals were sampled in triplicate at the beginning of the first colonization period.*

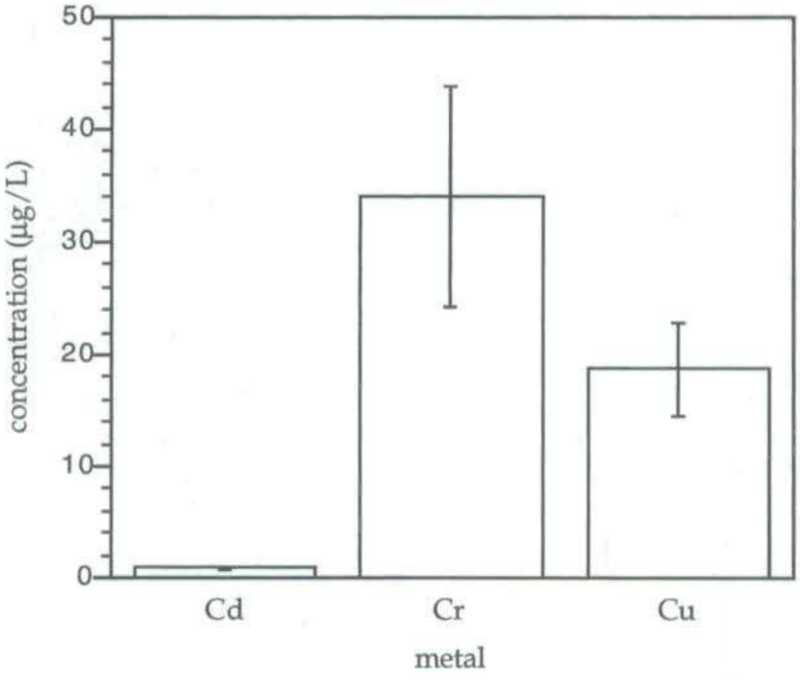


Figure 3.2 *Metal concentrations (µg/l) in water from Reshotka River (September 1997) based on analysis of triplicate samples.*

3.2 Metal concentrations and other parameters in sediment

3.2.1 Total metals in sediment

From the results of the analysis of total metals in sediment, it is clear that Cr has accumulated in the sediments of the Chusovaya's tributary, as well as at the downstream sampling site. Levels at the downstream site (2438 ± 207 µg/g) were more than 20 times higher than at the upstream site (101 ± 3 µg/g). At the mouth of the tributary, Cr levels were more than 50 times higher than at the upstream site (Figure 3.3). Cr is clearly the main contaminant in the sediments. Most other metals analyzed were not higher than background levels. However, Ni concentrations were slightly elevated at the downstream site (274 ± 25 µg/g compared to 103 ± 4 µg/g upstream), probably due to the input from the tributary, where the levels were the highest (413 ± 43 µg/g). Sediments from both upstream and downstream sites on the Chusovaya had similar Cu levels. Sediments from the tributary had slightly lower Cu concentrations compared to these two sites.

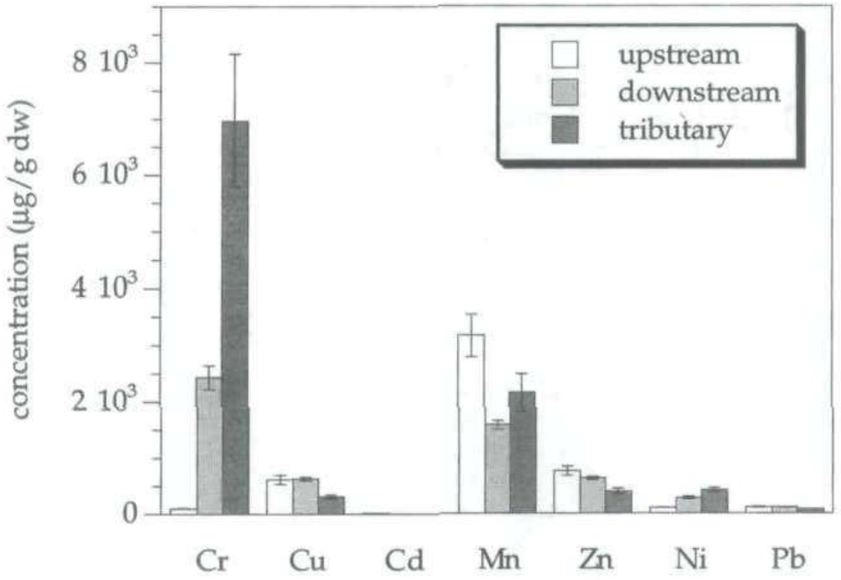


Figure 3.3 *Metal concentration (µg/g dw) in sediment after acid extraction of total metals for the study sites on the Chusovaya River and its tributary (Pakhotka River). (n = 9)*

3.2.2 Bioavailable metals in sediment

The mild extraction procedure which is meant to approximate the bioavailable (exchangable) metal fraction in the sediments would indicate that most of the Cr in these sediments is not bioavailable, although there is significantly ($p<0.05$) more Cr downstream than upstream in this fraction (Figure 3.4). The pH of extracts before addition of HNO_3 was in each case near neutrality (7.0 ± 0.041 for upstream, 7.20 ± 0.018 for downstream, and 7.66 ± 0.022 for the tributary samples). These results indicate that a greater proportion of the Cr in the tributary is bioavailable than in the Chusovaya. For bioavailable Cr, the tributary:downstream site ratio was only about 15:2. For total Cr concentration, this ratio is closer to 50:2. The differences in Cu concentrations among the sites was marginal for the bioavailable fraction.

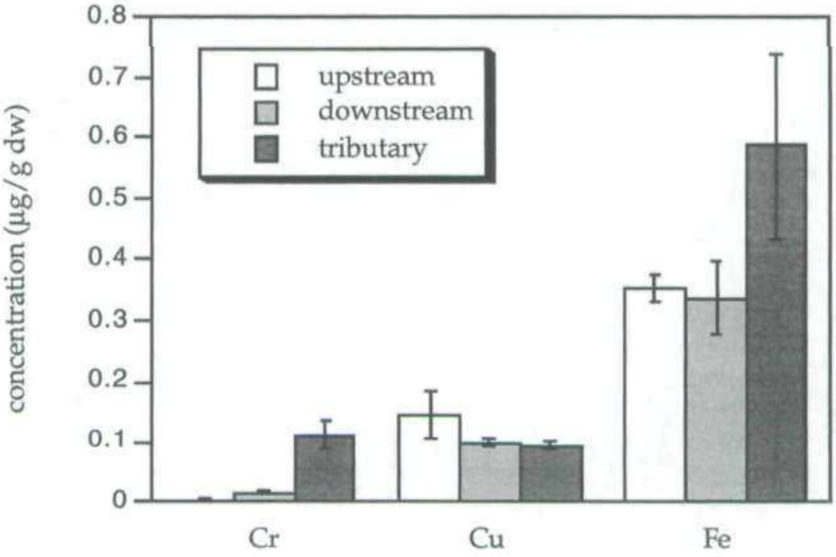


Figure 3.4 *Metal concentration (µg/g dw) in sediment after mild extraction of metals with $CaCl_2$ for the study sites on the Chusovaya River and its tributary (Pakhotka River). (n = 9)*

3.2.3 Grain size, total carbonate, and organic matter

The grain size distribution analysis showed that generally, the more polluted the site, the larger the grain size tended to be. The <63 µm fraction was the largest at the upstream site, 68.7 ± 1.7 % (w/w), followed by 53.2 ± 5.4% (downstream) and 24.1 ± 4.0% (tributary) (Figure 3.5). The tributary sediments were clearly sandier, where the <210 µm fraction made up 41.1 ± 5.0% by weight compared to the downstream site (79.7 ± 1.5%) and upstream site (82.6 ± 1.2%).

The total carbonate concentration was lowest at the upstream location (7 ± 0.8 g/kg) although the downstream location was not much higher (11 ± 1 g/kg). The tributary sediments were significantly richer in carbonate (36 ± 2 g/kg) (see Appendix IV).

The upstream site was the most enriched with organic matter while there was no significant difference between the organic matter in sediments from the tributary and the downstream site (Figure 3.6).

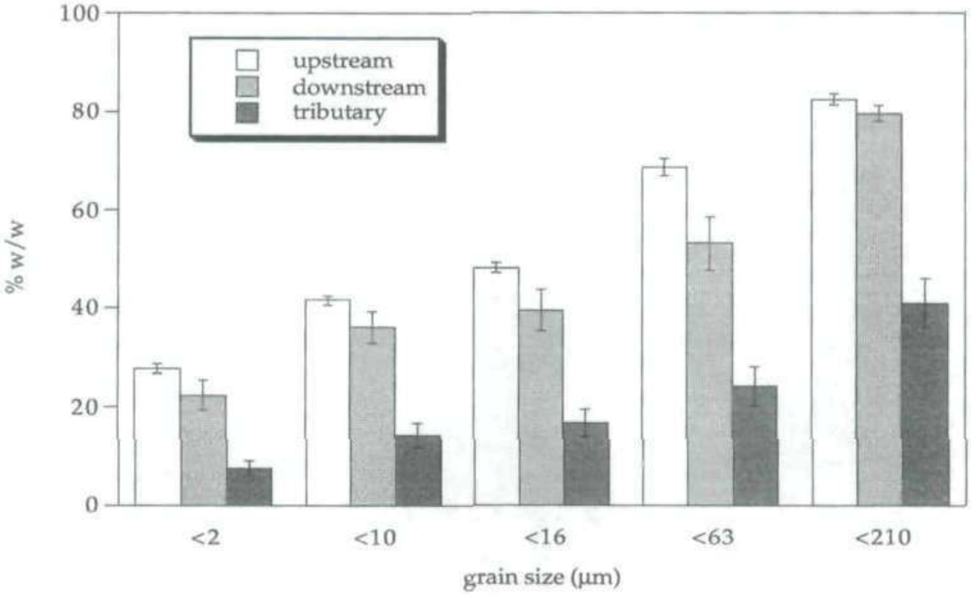


Figure 3.5 *Grain size distribution at upstream and downstream sites on the Chusovaya River and in its tributary (Pakhotka River) expressed as % w/w. (n = 9)*

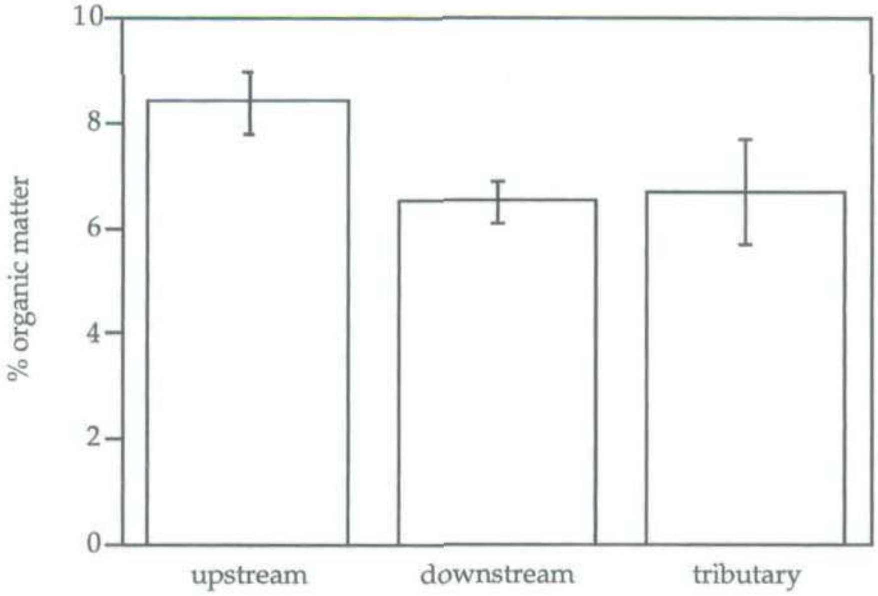


Figure 3.6 *Organic matter content of sediments from upstream and downstream sites on the Chusovaya River and from its tributary (Pakhotka River) expressed as % of total weight. (n = 9)*

3.3 Metal concentrations in detritus

The trend of elevated Cr levels found in water and sediment from the polluted site was also observed in detritus. Analysis of detritus collected from the site downstream of the tributary showed approximately 165 times the Cr concentration of the detritus collected at the upstream reference site (Table 3.1). The Cu concentrations in detritus from both sites were on the same order of magnitude, although higher at the upstream site. The Cr concentrations in the standard (NIST 2704) sediment run *in triplo* in the same extraction procedure showed consistently lower Cr concentrations than the reported certified values. (Observed concentrations were 54, 57 and 57% of the certified values for Cr.) For Cu, also tested in the three standard extracts, the recovery was much closer to certified values, 97% (Cu).

Table 3.1 *Metal concentrations in detritus from the upstream and downstream sites on the Chusovaya River, n=9 per site (concentrations in μg/g dw).*

SITE	Cr	Cu
upstream	102 ± 9	1375 ± 48
downstream	16870 ± 798	976 ± 20

3.4 Benthic macroinvertebrate communities

Species lists of the benthic macroinvertebrates collected from the colonized artificial samplers show that the downstream site was degraded. No species were deteced in the tributary. Application of the Shannon diversity index to the data showed that the diversity was high at the upstream site, (H' = 4.6) and indicates that the water quality is good (Shannon & Weaver, 1949). At the downstream site the number of different species identified was 55 % of the number of species upstream (52 compared to 94). The Shannon index value was lower (H' = 3.1) at the downstream site (Table 3.2).

The index of Margalef (which is not dependent on the number of individuals per species, but rather on the total number of individuals and the total number of species present) also indicated that the upstream site was very species rich (index value = 12.3), *indicating much greater diversity than the downstream site (5.9) (Table 3.2).*

The application of the ITC index indicates the absence of trophic groups throughout the summer (Table 3.2). Although there were no samplers placed in the polluted tributary in 1997, a total absence of benthic organisms was observed there after examination of riverbed samples taken with a circular shovel. This indicated that there is no change in the situation since the previous attempts to colonize artificial substrates there ailed. The tributary remains uninhabitable for benthic macroinvertebrates. The trophic groups continuously missing from the downstream site were numbers 4 and 11 (both herbivorous). According to the ITC, the functional structure at the upstream site was also in a disturbed state, where trophic groups 3, 4 and 11 (herbivorous) were also missing during the colonization periods (Table 3.2).

During the three different colonization periods (roughly the months July, August and September) the representation of the trophic groups fluctuated at both sites. In addition to trophic groups 4 and 11, at the downstream location trophic groups 10 and 12 were missing in the first (July) colonization period (Table 3.2). In August, groups 10 and 12 were again present, but group 5 was not represented. In September, representatives of group 5 were also detected, but groups 8 and 12 were missing.

Upstream, in addition to the fourth group being absent, during the first (July) colonization period, groups 3, 11 and 12 were also absent. In September, even more groups were not detected, namely 3, 5, 9, 11, and 12. The August colonization period was the most rich in trophic groups, with only the groups 3 and 4 missing. Downstream, August was also the month in which the most trophic groups were represented.

Table 3.2 *Comparison of values for the Shannon and Margalef diversity indices with the functional approach Index of Trophic Completeness (ITC) for communities of benthic macroinvertebrates at the Chusovaya River study sites. Missing ITC trophic groups are given per colonization period (1,2,3), Data was pooled from three colonization periods during the summer of 1997, and black wedges in ITC pie graphs indicate missing trophic groups (maximum 12 trophic groups).*

	upstream	downstream	tributary
Shannon index summer 1997	4.6	3.1	0
Margalef index summer 1997	12.3	5.9	0
missing ITC trophic groups colonization period 1	 **3**, **4**, **11**, 12	 **4**, 10, **11**, 12	
missing ITC trophic groups colonization period 2	 **3**, **4**, **11**	 **4**, 5, **11**	
missing ITC trophic groups colonization period 3	 3, **4**, 5, 9, **11**, 12	 **4**, 8, **11**, 12	
ITC groups not detected during sampling program	 **3, 4, 11**	 **4, 11**	 **1** to **12**

3.5 Bioaccumlation of metals in *Hydropsyche pellucidula*

The caddisfly larvae collected from the downstream polluted site showed a 40-fold increase in Cr concentration (μg/g dw) compared to conspecifics collected from the relatively clean Reshotka River (Figure 3.8). Cu was also somewhat more concentrated in larvae from the downstream site, consistent with the slightly elevated levels of Cu in the water. There was no detectable correlation between accumulation and body length, colour of exoskeleton, or other abnormalities in gills and anal papillae. The Cr bioconcentration factor of the organisms from the polluted site was 61.2 ± 3.1 compared to 41.9 ± 2.6 for organisms from the reference site (Reshotka River). Cu was also accumulated in the larvae, but to a much lesser extent (2 times) compared to the Cr bioaccumulation (40 times).

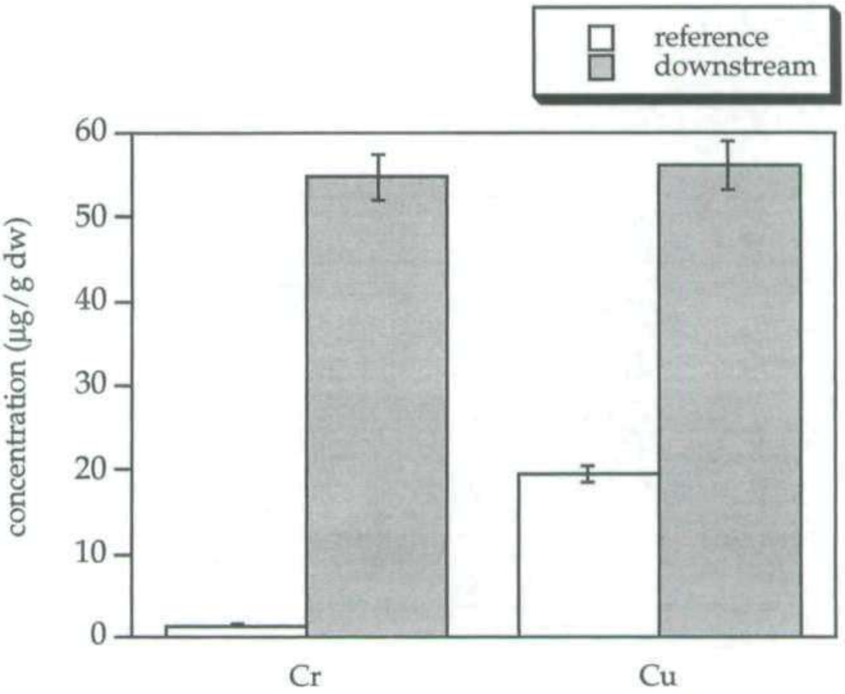


Figure 3.8 *Cr and Cu concentrations in* H. pellucidula *larvae collected from the Chusovaya River downstream site (n=19) and the clean Reshotka River (n=20).*

3.6 Abnormalities in *Hydropsyche pellucidula*

Fifth larval instars of *Hydropsyche pellucidula* from the clean reference site on the Reshotka River showed low levels of the physical abnormalities screened in this study. Tracheal gills were in reasonably good condition in all 20 larvae examined. The individual which had the most gill damage also appeared to be suffering from an extensive fungal infection, which may have aggravated the gill problem. The 14 fifth instar larvae from the downstream site on the Chusovaya suffered from more gill damage than conspecifics from the Reshotka, and the average severity according to the scale described in section 2.8 was significantly ($p<0.05$) higher (Table 3.3).

Most of the individuals from the Reshotka River had anal papillae which were either clear or white in colour, described by Vuori (1994) to be a normal field observation for this genus. Six percent of the larvae showed some sign of discolouration in the anal papillae. The most minor case was a dark ring around one of the four papillae, while in the most severe case, two of the four papillae exhibited darkening. The anal papillae of larvae from the Chusovaya downstream site were more severely affected, both in the number of papillae per individual which showed signs of darkening, and in the total number of individuals affected. A comparison between the two populations shows that a much greater percentage of the individuals from the downstream population exhibited discolouration of the papillae than from the reference population in the Reshotka (Table 3.3).

Table 3.3 *Occurrence of anal papillae discolouration (% of population affected) and level of tracheal gill damage (average value of damage scale, see text) in* H. pellucidula *larvae.*

SITE	average value tracheal gill damage scale	% anal papillae discolouration
reference	0.9 ± 0.2	20
downstream	1.8 ± 0.2	80

4.0 DISCUSSION

4.1 Chemistry

The total Cr concentrations in the Chusovaya at Pervouralsk exceeded the Russian toxicological norm (Gosudarstvennyj komitet po okrane prirody, 1990) in the summer of 1997 by 92 times. In 1995, the total Cr at the downstream site was even higher (1800 μg/l) than in the present study; in 1994, total Cr was 700 μg/l (Pashekevich & Pavluk, 1996). This river system is clearly subject to severe chronic chromium stress, even when compared to other heavily polluted rivers. Renoldi *et al.* (1997) reported Cr concentrations in water of the Lambro River (polluted tributary of the Po in Northern Italy) of 18.1 μg/l (dissolved) and 12.0 μg/l (particulate), *i.e.* total = 30 μg/l. Although the Cr enrichment was 18 times higher than background levels, this is still considerably less elevated than the levels found in the Chusovaya River.

In this study period, the Cr concentration in water at the upstream site was comparable to natural Cr background concentrations for surface waters in the Netherlands (Slooff *et al.*, 1989) or Canada (Pawlisz *et al.*, 1997) or Northern Italy (Renoldi *et al.*, 1997) and is equal to or under the values for norms in Russia (Gosudarstvennyj komitet po okrane prirody, 1990), the Netherlands (Evaluatienota Water, 1994), and Canada (CCME, 1987).

In this study, it was important to have similar sites in order to assume that any differences were the result of specific pollution differences, and not general water quality differences. The results of water quality analyses support the choice made. Besides Cr concentrations, current velocity was the only important difference between the sites, and this most likely had implications for the number of trophic groups present upstream. It seems to have certainly affected the density of hydropsychids present. Upstream only *H. pellucidula* were found, and the densities were much lower than at the downstream location, where hydropsychids were relatively abundant. (*H. pellucidula* is a species that is unusual in its genus for tolerance of slow current velocity (Hicken, 1967), which could explain its presence upstream.) Although species richness was still very high at the upstream site, the ITC index detected a disturbance. This is likely the result of the river engineering which greatly affected the current velocity at this site.

Though some of the other trace elements are present at the downstream site at concentrations slightly higher than background concentrations at the upstream site, all were low enough to comply with Russian drinking water quality criteria (Gosudarstvennyj komitet po okrane prirody, 1990).

The study sites on the Chusovaya (as well as the site where reference *H. pellucidula* were collected on the Reshotka) are all very aerobic and the calculated estimations of Eh were very high. High Eh at the measured pH range for the waters in this study is common to most natural running waters (Baas Becking *et al.*, 1960). Thermodynamic calculations indicate that under conditions of high Eh and a pH between 7 and 8, Cr should exist primarily in the hexavalent form (see Figure 4.1). However, the speciation seems to be to some extent dependent on other factors as well (Pawlisz *et al.*, 1997; Cranston, 1983). In a previous study of the Chusovaya River, Cr(VI) did not account for all of the Cr found present. Because of the low oxidation rate of Cr(III) to Cr(VI), (in oxidizing water, it may take up to 30 days for Cr(III) to be oxidized) it is possible that despite the thermodynamic instability it should have, some Cr(III) may be present in an oxidized water column (Shroeder & Lee, 1975). In this case, it would probably be present as $Cr(OH)_3$ (Stumm & Morgan, 1981). At the Chusovaya downstream site, we therefore expect Cr(VI) (present as CrO_4^{2-}) to make up a considerable proportion of the total Cr measured, with Cr(III) being present predominantly as $Cr(OH)_3$. The presence of the bioavailable form of Cr is demonstrated by Cr bioaccumulation, and other effects in caddisfly larvae and the absence of species and funtional groups.

Cr(III) is quite insoluble in water and will strongly adsorb to the negatively charged surfaces of organic matter or (clay)minerals. Particularly at higher pH, the hydroxy functional groups on organic particles will become deprotonated, increasing adsorption sites for cations. Cr(VI) compounds are able to adsorb to positively charged surfaces such as iron, manganese or aluminum oxides and hydroxides, but this adsorption is limited at the alkaline pH in the Chusovaya. Furthermore, there are two other expected fates for Cr(VI) in sediments besides adsorption, and they are reduction to Cr(III) or transport to the ground water. It is clear from the very high levels of Cr measured in the total metals extraction of sediments of the tributary that adsorption of Cr has taken place in the sediment. Considering that the trivalent state of Cr is generally more stable in sediments, and that free Cr(VI) in sediments would not likely have a long residence time because it can be quickly transported to groundwater, the trivalent species is probably responsible for most of the Cr concentration measured in the sediments at the downstream site.

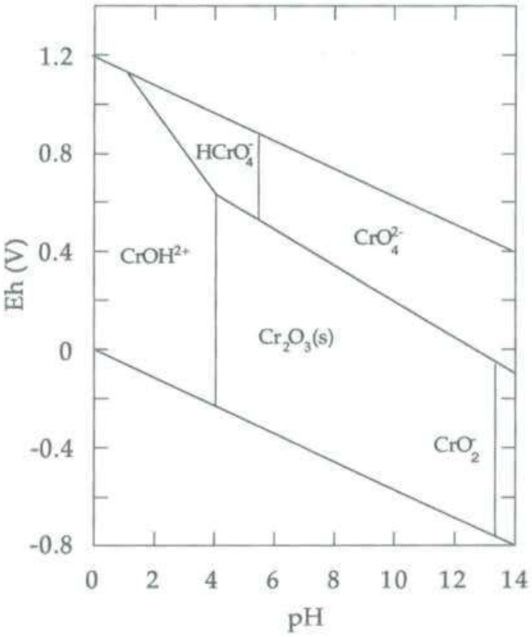


Figure 4.1 *An example of an Eh-pH diagram for chromium (for $[Cr]_{dissolved} = 10^{-6}$ M and 25 °C). (Top line and lowest line indicate resp. upper and lower limits of water stability.) After Sigg and Stumm, 1994.*

Besides mineral surfaces, organic matter, such as humic substances (products of organic matter decomposition) can provide a large amount of binding sites for metal ions such as Cr(III) (Bidoglio *et al.*, 1997). The complexes resulting from adsorption to organic matter, especially humic substances can be very stable (Hellawell, 1986) and also not generally very bioavailable. This may not always be the case however, as some authors suggest that under acidic conditions in humic waters may increase the lipid solubility of humic substances and the associated metals, making them more able to pass membranes (Petersen *et al.*, 1987, Penttinen *et al.*, 1995). Therefore, the presence of humic substances is an important parameter to consider in any study of the fate of Cr. In a study of Finnish surface waters (Tarvainen *et al.*, 1997), the highest concentrations of Cr were found in brown stream and lake waters rich in humic matter. The binding of Cr(III) to humic substances in the water column can facilitate the transport of this species (Bidoglio *et al.*, 1997), which otherwise has the tendency to precipitate out of the water column. In addition, the formation of complexes strongly inhibits potential oxidation processes (Cotton & Wilkinson, 1980). Humic substances associated with sediments will act to keep Cr in this environmental compartment. In the stained water of the tributary in the present study, the Pt-Co colour scale value was more than 10 times higher than the value for the upstream site, suggesting a high concentration of humic substances, or possibly interference from the high level of Cr present. Cr compounds are known for their pigmentation properties, (chromates (CrO_4^{2-}) are yellow, Bailar *et al.*, 1973), which if present, may be a factor which interferes with the Pt-unit analysis. The colour value at the downstream site was approximately double the upstream site, and if this is due to humic substances, (which in principle could easily be present in the slightly alkaline waters), it would indicate a stronger metal binding power at the downstream site. The colour difference at the downstream site appears to be the result of the influence of the tributary. The exact cause of the colour difference should be further investigated because the binding of Cr to humic substances will have an effect on the transport of Cr contamination to locations further downstream on the Chusovaya River.

Besides humic substances, other parameters which are important for the redox state of Cr are the presence of compounds which reduce Cr(VI) to Cr(III), such as Fe(II), (Slooff *et al.*, 1989) and low molecular weight organic compounds and proteins. There was no Fe(II) found, and the Sr levels (another reductant) measured in an earlier study (Stepanov *et al.*, 1992) were low. The (top layer of) sediments sampled in this study did not have a strongly anaerobic, reducing character, and this would not enhance the reduction of Cr(VI) to Cr(III).

For benthic communities living on natural substrates, the sediment quality is very important because of the direct contact they have with this phase. Particularly organisms such as Chironomidae and Oligochaeta live in very intimate contact with sediments and the interstitial waters. In general, contamination in sediment may also be released to the water column under certain conditions, which makes sediment contamination in general a potential risk for water quality. In the case of Cr, this is expected to mainly be the result of resuspension of particles to which Cr is adsorbed, followed by desorption and release of Cr in the water column: diffusion down a gradient to relatively Cr-free water is not considered likely (Slooff *et al.*, 1989). Sediment quality data for Dutch aquatic sediments reviewed in the same report show that after Cr concentrations in surface waters decreased, the Cr levels in sediments also dropped (after a lag time).

In this study, the total Cr concentrations in sediment were much higher downstream than upstream with a maximum at the mouth of the tributary, reflecting the concentrations of Cr in water. The background levels of Cr observed at the upstream site were very close to 100 μg/g dw which is similar to values from the literature as reviewed in Slooff *et al.* (1989) (*e.g.* aquatic sediments in the Netherlands before 1880 were ca. 70 μg/g, and 40 (NAS) to 100 μg/g (EPA) have been reported). The norm for Dutch aquatic sediments is also 100 μg/g dw (Evaluatienota Water, 1994). The average Cr concentration in aquatic sediments in the Netherlands has been determined to be currently between 15 and 30 μg/g (Slooff *et al.*, 1989). The region around the Chusovaya River is quite rich in serpentine, a mineral rich in Cr which can lead to naturally elevated background levels of Cr, although it is probably not a very significant factor in this case. In another study done on sediments in the Chusovaya River (Stepanov *et al.*, 1992), the metal concentrations found in the sediments near the downstream site in this study (which they refer to as "hard packed," roughly meaning small grain size) were consistently lower than the findings in this study. (However, the method of metal extraction is not reported, and the analysis was performed on one sample only.) A Cr content of 1604 μg/g dw was measured, compared to 2438 ± 1999 μg/g dw in this study.

Field studies involving the assessment of the impact of Cr point sources on the aquatic ecosystem are rather scarce, although one recent study in the Netherlands included Cr in the series of contaminants to be investigated in river sediments. The Cr levels measured in the Chusovaya are considerably higher than those detected in the study of the biotic effects of contaminated aquatic sediments of the River Nieuwe Merwede (Rhine River Delta), where the Cr concentrations ranged from <24 to 430 μg/g dw, and was accumulated by the freshwater mussel *Corbicula* (2.6-12.6 μg/g dw) (Den Besten, 1993). Heavily Cr contaminated sites are also found in Canada, for example, the Welland Canal (the most contaminated site in the province of Ontario), downstream from a steel plant where sediment Cr concentrations exceeded 5120 μg/g dw (Dickman *et al.*, 1990). This is comparable to the tributary in the present study. Cr concentrations in sediments near industrial effluent inputs have not often been reported to exceed this value (Pawlisz *et al.*, 1997), although perhaps the most extreme case of Cr contamination known is in the St.

Mary's River system where sedimentation of chromium in "Tannery Bay" has resulted in levels reported to be as high as 31000 µg/g (Environment Canada, 1988).

The $CaCl_2$ extraction of sediments (the assumed bioavailable fraction) indicated that hardly any of the Cr present in the sediments belongs to the bioavailable fraction, under the extraction conditions. Any Cr(III) bound to river bed components (*i.e.* sediment grains, organic material, or humic substances) must have been strongly adsorbed, and were not released in this mild extraction method. The low levels of Cr detected did follow the same pattern as the results of the total metals extraction, with the highest levels in the tributary, followed by intermediate levels downstream and very low levels at the upstream site. The Cr measured in these extracts could well have been (anionic) Cr(VI) which was present in dissolved form in the interstitial water associated with the sediments. It would seem likely that it is mainly the Cr contamination in the water that precludes life in the tributary, and that the strongly adsorbed Cr contamination in the sediments is not a serious risk to benthic organisms under the current conditions.

Despite the larger grain size and lower organic enrichment than upstream, (and thus a lower capacity to trap and hold metals in sediment), the Cr concentrations were still higher downstream, and even higher in the tributary. The concentration differences between sites would be even greater if calculations were adjusted for these factors.

The results indicate that a greater proportion of the Cr in the tributary is bioavailable than in the Chusovaya. For bioavailable Cr, the tributary:downstream site ratio was only about 15:2. For total Cr concentration, this ratio is closer to 50:2. This suggests that the bulk of the total Cr in the sediments is in the trivalent form.

The high concentration of Cr in detritus from the downstream site can be explained by the binding of Cr(III) that adsorbs to these small particles with large surface to volume ratio. Also, this plant material may have accumulated Cr during the life of the plant. Large increases in Cr concentration have been found in living plants collected from the same downstream site. Stepanov *et al.* (1992) measured metals in sedge, both roots and above water stems, from the Chusovaya at Pervouralsk, and also at an upstream location near the upstream site in the present study. Upstream they measured 33.3 and 1.06 mg Cr/kg dw for roots and stems respectively, compared to 2229 and 8.3 mg Cr/kg dw in plants collected at the downstream site. Especially roots were found to accumulate Cr, a possible indication that there is indeed bioavailable (hexavalent) Cr in the sediments, which may be quickly assimilated by plants before reduction to Cr(III) can take place.

According to a comparison of digestion techniques (Cook *et al.*, 1997), the amount of metal extracted tends to depend on the extraction technique employed (strong acid or the more aggressive HF normally employed for the determination of certified values in standard reference material). The results of the study, in which some 20 labs participated, showed that the choice of digestion method generally did not affect the recovery of Cu, while for Cr the HF extraction recovery was between 15 and 70% higher compared to the recovery with strong acid extraction. The concentrations of Cr measured in detritus were not adjusted and the digestion was considered to be successful, because of the excellent recovery of Cu, and despite the low recovery of Cr. The most important aspect in this study is the comparison of the sites. Since the efficiency was the same for all samples in the digestion, this may be done despite the low recovery from the strong acid digestion technique employed.

To improve on these recoveries, standard reference material should be chosen as close as possible in identity to the material being extracted, large masses should be used in the extraction to prevent effects of heterogeneity. If necessary, more aggressive extraction procedures may be employed.

4.2 Benthic macroinvertebrate communities

4.2.1 Sampling considerations

Many different sampling methods exist for benthic macroinvertebrates, such as shovels, dredges, hand nets (see Hellawell, 1978 for review). The method using Dutch Artificial Substrate Samplers (Bij de Vaate and Greijdanus-Klaas, 1990) was chosen because of the standardization of sampling it provides compared to other methods (Pashkevich *et al.*, 1996). This means that comparisons between rivers, or between different sites on the same river can more easily be made because parameters such as grain size, which these animals can be particularly sensitive to, do not play a role in determining the species which are likely to be present. Generally, the main factor determining which species will be present is the toxicant concentration of the river water. In addition, this method is an efficient way to collect a large amount of animals in a relatively short amount of time (compared to circular shovel sampling for example).

4.2.2 Community structure

Effects on the trophic structure of the benthic macroinvertebrate community were visible at both sites on the Chusovaya. Upstream, the trophic structure was not complete, despite the high species diversity. This can be attributed to the low current velocity (0.1 m/s) observed at this site over most of the summer, due to river engineering in the vicinity of the town of Revda. Drinking water for Revda is tapped from the Volchikhinsky reservoir just upstream from this site, which undoubtedly affects the current velocity at the study site. Further downstream after the confluence of the Revda River with the Chusovaya (in between the upstream and downstream sites) the current velocity increases again. This suggests that the ITC method is also able to detect effects of physical changes in the aquatic environment.

Downstream, there were three to four trophic groups missing per colonization period, including the groups 4, 11, (always absent) and the groups 5, 8, 10, and 12 (absent in up to two of the three colonization periods). In a previous study, (Pavluk, 1997) this downstream site had four trophic groups missing (numbers 4, 5, 11 and 12). Number 5 (small herbivorous animals which crush vegetative material <1 mm) appears to have made a comeback since 1995, but the individuals representing this groups were not abundant and not present during all colonization periods. Groups 8 and 10 showed sensitivity to the conditions at this site. This testifies to the fluctuations in water quality, although other factors, such as discharge (which also can indirectly affect water quality), also influence the structure of the community.

The trophic groups 3, 4, 11 and 12 are all functionally connected to macrophytes and plant residues (Pavluk, 1997). Group 3 consists of omnivorous animals and is also functionally connected with zooplankton and macrobenthos. Group 11 is made up of herbivores who depend on "the liquid contents of vegetative cells" as a food source and are therefore functionally connected only with macrophytes and plant residues (Pavluk, 1997). Group 12 animals are omnivorous "microfeeders" (food <1 mm), and according to Pavluk are functionally connected to microbenthos in addition to macrophytes and plant residues. Pavluk places herbivores which feed on large (>1 mm) plant material in group 4, connected to periphyton and macrophytes/plant residues. The groups chronically missing from the Chusovaya River all have in common that they are functionally connected to the macrophytes and plant residues compartment of the aquatic ecosystem.

An effect on the macrophyte community can translate into stress for animals which are wholly or even partly dependent on this food source. It may be that the absence of the trophic groups was the result of disturbance to the macrophyte community. At the upstream site, macrophytes were more abundant. The possibility that at the upstream location the effect is observed due to current velocity and at the downstream location is in part connected with a disturbance of macrophytes and plant residues thereof should be further investigated. However, another important factor is the possible influence of the sampling method. Artificial substrates (where the macrophyte food source is absent), may be less attractive to (partly) macrophyte-dependent groups than other trophic groups.

The ITC method was able to detect a disturbance of the upstream site, which suggests that it is sensitive to reduction of current velocity. It is unable to clearly distinguish between the Cr disturbance downstream and the velocity disturbance upstream, although it is better able to provide insight into the reasons behind the disappearance of functional groups than the other indices calculated, which give information only on the general diversity of species present. Both diversity indices could show that the diversity is higher upstream than at the downstream site, as is expected considering the absence of Cr contamination in the water and the lack of sensitivity of diversity indices for changes in current velocity.

The variation in the number of trophic groups represented in the Chusovaya River at both sites during the summer of 1997 can be attributed to changes in water quality or other physical parameters such as temperature and current velocity. The largest number of trophic groups were detected in the August colonization period, when water quality was not strikingly different from other periods, but during this period at the upstream site, the current velocity suddenly increased due to the opening of the locks at the upstream reservoir. This is likely to have been the most important factor which negatively affected the number of missing groups at this site during the August colonization period. In August, the rainfall was also very high, and the resulting conditions of high water at the downstream site would have diluted the Cr input from the tributary, perhaps making it possible for some groups (such as group 5) to temporarily return.

4.3 Ecotoxicology

Considering that in water, sediment and detritus elevated concentrations of Cr were observed in all samples from polluted downstream site, benthic macroinvertebrates from this site were exposed to Cr potentially via several different routes. Caddisfly larvae of the species *Hydropsyche pellucidula* live under stones and catch prey in spun nets. These filter feeders are mainly exposed to contamination in the water column and seston (Vuori, 1996). In the winter, a significant part of their diet consists of detritus. Therefore these two compartments, water and detritus would be the most important in terms of direct exposure. Of the total Cr in the water column, it is likely that both Cr(VI) and Cr(III) are present. Cr(VI) would be the most bioavailable form, being dissolved and having the ability to easily pass cell membranes. Cr(III) would be the less bioavailable form, strongly bound to humic substances, or to suspended particles (although the particulate fraction of the water is very small, appendix I). The Cr(VI) is the form that can easily be assimilated, and then reduced to Cr(III) in cells where it accumulates and exerts its toxic effects.

The increased incidence of anal papilla discolouration in *H. pellucidula* from the downstream site is an indication of the effect of the main pollutant, Cr, on these organs. These ion exchange organs are responsible for taking up ions out of the aquatic environment and ensuring that the hypertonic body fluids of the animal remain hypertonic with respect to the surrounding water. For this reason, one would be expect them to be easy target organs for Cr(VI). Once past the membranes, this species is reduced to the insoluble trivalent form, which has the ability to bond to biological macromolecules such as proteins and DNA in the cell. In the cell, the insoluble Cr(III) is likely very difficult to excrete again, and may be accumulated. The metal accumulation may thus be the cause of the darkening effects visible on the anal papillae. Vuori (1994, 1996) has previously reported damage to anal papillae and metal accumulation (metals investigated: Cd, Cu, Zn, Fe, Al). In the present study, the same effects have now also been identified in the Cr contaminated Chusovaya River. Vuori's results (1996) suggest that there is a correlation between Al accumulation and the discolouration observed in *H. pellucidula* he collected from an Al-polluted Finnish river (where Al concentrations ranged from 759 to 6500 μg/l during sampling). This correlation was also observed in laboratory Al-exposure tests (Vuori, 1995). In the present study, there is also correlation between Cr accumulation and abnormalities observed in *H. pellucidula* on population level.

The anal papillae endpoint is not only a tool to screen for damage caused by toxicants such as metals, or even chlorine (Camargo, 1991) but it also has an ecological significance because papillae damage reduces the fitness of affected individuals. Gill damage is equally significant for the health and survival of the individual, as well as being an indicator of contamination effects. It has previously been suggested that hydropsychids have potential as bioindicators of trace elements in lotic systems (Cain *et al.*, 1992; Petersen, 1986; Higler & Tolkamp, 1983). The findings of the present study and those of Vuori (1992, 1994, 1996) demonstrate that the described physical abnormalities in hydropsychids are indicators of metal pollution, and considering the ease of collection and examination of hydropsychids, they are recommended bioindicators for river systems.

Bioconcentration factors are dependent on the form in which the toxicant is present (which affects its bioavailability), and the ability of the organism to avoid uptake or to excrete it. The data here show that *H. pellucidula* larvae are not able to regulate their Cr uptake effectively when the concentrations are as high as in the Chusovaya. In the tributary, the Cr level is too toxic for survival of macrozoobenthos. At the relatively low Cr concentrations at the upstream site, the bioconcentration factor was a third lower than at the downstream site, indicating that the net uptake is better regulated at the lower concentration.

No data were found in the literature on the effects of Cr on hydropsychids except for a Cr(III) bioassay with *H. betteni*, which was found to be tolerant up to very high concentrations (96h LC50 of 64 mg/l) (Warnick & Bell, 1969). Invertebrates are generally more sensitive to elevated Cr levels than fish (Pawlisz *et al.*, 1997; Hellawell, 1986). Of the freshwater invertebrates reviewed in Pawlisz *et al.* (1997), the crustacean *Daphnia magna* is the most sensitive (64 h EC50 of 1200 μg/l) (Anderson, 1950). Another invertebrate sensitive to Cr(III) is the mayfly *Ephemerella subvaria*, for which the 96 h LC50 was 2000 μg/l (Warnick & Bell, 1969). Mayfly diversity was reduced at the Chusovaya downstream site, where total Cr was nearly half this concentration. This may be due to sensitivity of mayflies to chromium (Pawlisz *et al.*, 1997).

Cr(VI) on the other hand, induces effects in invertebrates at much lower concentrations. For example, the 24 h EC50 (immobilization) of 53 μg/l for the daphnid, *Ceriodaphnia dubia* (Hickey, 1989). This is a very low concentration compared to what is observed at the Chusovaya downstream site. At concentrations in the range measured in the Chusovaya, a reduction in fecundity is observed in the mollusc *Biomphalaira glabrata* under chronic exposure to 1400 μg/l hexavalent Cr. *Tubifex tubifex* (annelid) is more tolerant of Cr(VI), and at 10000 μg/l experiences acceleration of the respiration rate (Brkovic-Popovic & Popovic, 1977). The chironomid *Chironomus tentans* shows a decrease in rest time only upon chronic exposure to extremely high levels of Cr (1000 mg/l) (Batac-Catalan & White, 1983).

Other animals which are at potential risk of the Cr contamination at the Chusovaya are local cows, which graze in the pastures adjacent to the heavily Cr contaminated Pakhotka and Chusovaya rivers, drinking the water. Hepatoxicity has observed in cattle as a result to Cr(III) body burdens over 9.6 mg Cr/g (Kreuzer *et al.*, 1985). Interim water quality guidelines for the protection of livestock species have been set at 50 μg/l for total Cr in Canada (Pawlitsz *et al.*, 1997).

4.4 Assessment Summary

The chromium contamination in the Chusovaya River affects the water, sediments, detrital material, and is accumulated by members of the benthic macroinvertebrate community. Total Cr concentrations are high, although the bulk of the Cr in sediments is likely to be in the trivalent oxidation state, and unavailable to the biotic component for assimilation. However, studies of the chronic effects of Cr(III) on invertebrates are lacking in the literature, and the potential risks of Cr(III) for this group cannot be properly assessed until this data gap is filled. In the water, the Cr is also present in the form that it is available to biota, as demonstrated by the reduction in species diversity at the downstream site, the absence of life in the tributary, and the bioaccumulation and physical abnormalities in caddisfly larvae. The contamination of the water is a major problem, since trivalent Cr in the sediments is not likely to become easily or quickly (re)oxidized to hexavalent Cr under normal circumstances. Complexation has the effect of stabilizing a system and preventing reduction (Stumm & Morgan, 1981). It has been reported that a large amount (>50%) of the total Cr in surface waters is involved in adsorption and complex-forming (Slooff *et al.*, 1989), making these processes very important for the mobility and transport of this trace element. This fact puts the Chusovaya River ecosystems further downstream from Pervouralsk also at potential risk.

The clear adverse effects on biota at the level of benthic macroinvertebrates are not expected to be transferred to higher levels of the food chain (fish, terrestrial insectivores) by biomagnification of Cr (Slooff *et al.*, 1989) since these vertebrates are not known to accumulate Cr to any great extent. However, adverse effects which change the benthic macroinvertebrate community structure and diversity cause changes to the foundations of the aquatic ecosystem. Effects of Cr may be translated to higher organisms in terms of reduction and lower diversity of food supply.

Assessments of the water, sediments and biota in freshwater systems under the influence of elevated Cr levels are few, and when Cr is included in the investigation, there are often many other contaminants present. Although there is a rather respectable amount of toxicological data on Cr (both tri- and hexavalent) to be found in the literature, there seems to be a paucity of published assessments of the effects of Cr point sources on freshwater ecosystems. The triad assessment described in this report has not only provided an indication of the ecological condition of the Chusovaya River at Pervouralsk, but it also represents an example of a unique *in situ* study of the impact of Cr river pollution in the absence of a contaminant mixture.

4.5 Ideas for future studies

Studies of problems involving Cr contamination are very complex because reliable information on Cr oxidation state is important for assessing the ultimate risk. Reliable and sensitive methods to analyze the relative proportions of Cr(III) and Cr(VI) in the total Cr should be investigated and used in the study of chromium contaminated river sites, since the redox predictions based on thermodynamic calculations are not always observed in nature due to the complexity of the processes affecting the redox state. The Chusovaya River provides an excellent *in situ* study site for further investigations of the fate and movement of Cr in a natural river system.

The observations of Cr bioaccumulation and adverse physical effects observed in this field study should be verified with laboratory testing with both Cr(VI) and Cr(III). Also, *in situ* tests may be designed with caddisflies, where the animals are placed in specially designed racks (Stuijfzand *et al.*, 1997) so that the residence time of the animals at the polluted site can be controlled. Chronic toxicity testing with invertebrates and Cr(III) is lacking in the literature, and this data gap should be filled considering this form may also be present to some extent even in well-aerated surface waters.

Hydropsychids are plentiful, relatively easy and inexpensive to sample and identify, and can indicate effects in ecologically significant endpoints (gill damage, anal papillae discolouration). This group has potential for biomonitoring purposes in Russia and elsewhere. If the contaminated site at Pervouralsk is remediated in the future, hydropsychids could be useful in monitoring improvements in the situation both in the Chusovaya River and in its tributary, the Pakhotka.

Further investigations into the sensitivity of hydropsychids throughout their lifecycle is useful, in particular to examine the effect of moulting on the uptake of metals. Also, the accumulation of metals in the anal papillae could be quantified and related to the overall body burden.

In studies with chironomids, genetically adapted populations have been identified at a metal contaminated site on the Dommel River in Belgium (Postma & Groenendijk, in press). The possibility of genetic adaptation to chromium could be further investigated in the Chusovaya River as well. Toxicity testing already has been carried out with a Cr-resistant strain of *Daphnia magna* (Munzinger & Monicelli, 1992), which raises the question of possible Cr adaptation in other species. Cr(III) is known to interact with DNA and other biological macromolecules in the cell. Cr(III)-DNA adducts could be screened in benthic macroinvertebrates from the Chusovaya.

It was clear from the investigations of the communities in the Chusovaya River that physical as well as chemical parameters are important factors influencing the structure of the benthic macroinvertebrate community. Further investigations into the effects of physical changes to the environment clearly are as important as chemical changes. A multivariate approach to the data analysis in studies of this kind is very useful in identifying the factors which are correlated to the effects observed. Control sites on other rivers in the Urals region, also upstream from point sources, were sampled during the same sampling period (summer 1997) as the Chusovaya in this study: the Iset River and Salda River (both Ob Basin). From initial analysis of the data, these sites are characterized by both high species richness and functional diversity (Pavluk, unpublished data). These sites may be compared with the upstream site on the Chusovaya in the future to gain further understanding of the impact of low current velocity.

CONCLUSIONS

- The Chusovaya River at Pervouralsk is heavily contaminated by chromium (Cr), and is characterized by elevated Cr concentrations in water, sediments and detrital material. Both hexavalent and trivalent Cr is expected to be present in this system, but in different proportions depending on the environmental compartment.
- The contamination in the water at the site has degraded the benthic macroinvertebrate community; contamination in sediments is not likely to be a direct risk compartment for aquatic life due to the apparent lack of bioavailability of the contamination there under the present conditions.
- The benthic macroinvertebrate community structure at the downstream site is degraded both in terms of species richness and functional (trophic) diversity.
- The benthic macroinvertebrate community structure at the upstream site is characterized by high species richness, but most likely as a result of low current velocity due to river engineering works, some damage to the trophic structure of the river community is observed.
- Larvae of the caddisfly *Hydropsyche pellucidula* (Trichoptera) bioaccumulated large amounts of Cr after exposure at the Cr contaminated downstream site.
- *Hydropsyche pellucidula* larvae from the Cr contaminated site also exhibited elevated incidence of the same physical abnormalities (tracheal gill damage and anal papillae discolouration) as previously observed in hydropsychids after exposure to other metals.
- Hydropsychids have excellent potential for biomonitoring in rivers because of their wide distribution and abundance and the possibility of using discolouration of anal papillae and gill damage as convenient endpoints.
- This triad assessment of a Cr polluted site demonstrated that the observed extent of Cr contamination had an adverse effect on the ecosystem in the Chusovaya River, both on community level (reduced diversity) and at the level of individuals (sublethal effects on surviving individuals).

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GLOSSARY

AAS - Atomic Absorption Spectrophotometry - a method to determine the concentration of a certain element in a (normally liquid) sample which is based on the principle that as an element is heated to atomization, the amount of light emission that is produced and absorbed is proportional to the amount of the element present.

acute toxicity - ability to produce a toxic effect (such as survival, growth, reproduction or behavioural reponses) after a short period of toxicant exposure (*e.g.* 48 or 96 h); (compare chronic toxicity).

anal papilla - retractible posterior ion exchange organs (4) of hydropsychids which are known to be sensitive to discolouration induced by dissolved metals in surrounding water

benthic macroinvertebrate - bottom-dwelling invertebrate in an aquatic system normally easily visible to the naked eye at somewhat advanced stages of development, *e.g.* molluscs, aquatic insect larvae, worms

bioaccumulation - process of (net) build-up of a chemical in an organism that is taken up (via the food or physical environment)

bioavailability - the degree to which a compound can potentially be assimilated or exert physiological and toxicological effects on an organism; in aquatic systems, influenced by water solubility, complexation and adsorption, pH, redox potential, speciation (metals), uptake kinetics characteristic of the organism themselves, etc.

bioconcentration factor (BCF) - ratio of the concentration of an element or compound in the body or tissues of an organism to the concentration of that element or compound directly available to the organism in the environment under steady state conditions.

bioindicator - a certain taxon which is focused upon to screen for adverse effects, and which can give an indication of the condition of the ecosystem and environment as a whole.

biomonitoring - the use of biological material to make a dinstinction between healthy and degraded systems, to trace the source of the impact, and to monitor the development after remediation measures have been taken; complements chemical monitoring programs by supplying extra information not to be gained by other monitoring approaches.

caddisflies - members of the family Trichoptera of the class Insecta, with aquatic larval stages (usually 5) and a terrestrial adult stage.

chronic toxicity - poisonous effect after a long period of toxicant exposure, sometimes defined as 90% of the lifetime; (compare acute toxicity).

detritus - plant material in some state of decompositon which in aquatic systems is used as food for benthic macroinvertebrates and is further mineralized by microorganisms; detritus fractions which are analyzed include associated microorganisms.

ecotoxicological endpoint - a characteristic of a test system that is the focus of observation in the study (*e.g.* incidence of deformities, mortality, accumulation)

Eh - see redox potential

electrolytic conductivity - a measure of the amount of electrolytes (dissociating solutes) in solution (units *e.g.* µS/cm)

exoskeleton - the chitinous outer covering of insects and crustaceans which gives form and structure to the bodies of these invertebrate organisms; exoskeletons are known to adsorb metals from surrounding solutions.

humic substances - polymers (300 to 30,000 MW) containing phenolic OH, carboxyl groups and aliphatic OH groups which are able to form complexes with metal ions; they are products of transformation of biological substances, and contribute to the bulk of organic matter in most water; classified as *a*) humic acid (soluble only in alkaline waters), *b*) fulvic acid (soluble over entire pH range), or *c*) humin (not extractible by acid or base). (Stumm and Morgan, 1981 p. 514)

hydropsychids - members of the caddisfly genus *Hydropsyche*.

ICP-AES - Inductively Coupled Plasma - Adsorption Emission Spectrophotometry.

loss-on-ignition - the loss of weight of an absolutely dry sediment sample after being at a temperature high enough to burn the organic matter present (*e.g.* 16 h, 375°C) and is thus a measure of the organic content of a sediment sample.

matrix modification - useful for analysis of certain metals (by AAS), it is the addition of a reagent (*e.g.* an acid or a salt) to a sample in high concentration in order to achieve better separation of the analyte element of interest by transforming it into a well-defined element with known properties.

moulting - the shedding of the exoskeleton between larval stages to make way for a new, larger exoskeleton that emerges from underneath the old exoskeleton.

Pt water colour scale - 1000 Pt units is defined as the colour from 2.492 g potassium hexachloroplatinate (K_2PtCl_6), 2 g cobaltic chloride hexahydrate ($CoCl_2.6H_2O$), 200 ml concentrated HCl and 800 ml water (Wetzel, 1975).

redox potential (Eh) - a measure of the degree of the oxidizing or reducing conditions in sediment (or soils); dependent on oxygen concentration and concentration of other oxidants in the system; a system with a high Eh is able to accept and transfer electrons, and is thus highly oxidizing; defined with the Nerst equation as follows:

$$Eh = E^\circ - RT/nF * \log([\text{oxidants}]/[\text{reductants}])$$

teratogenic effect - disturbance of developmental processes leading to structural or functional abnormalities as a result of prenatal (or egg stage) exposure of an organism to a teratogenic compound.

triad assessment - an assessment of the quality of an environmental system based on data on the chemical nature and extent of contamination, the structure of a community in the ecosystem (benthic macroinvertebrates), and ecotoxicological endpoints.

APPENDIX IA

WATER METAL CONCENTRATIONS - DATA

concentrations in µg/L

sample ID	sample date	Cr	Cu	Cd	Zn
RESHOTKA RIVER:					
Resh-1	08.10.97	40.5	12.3	0.998	51
Resh-2	08.10.97	46.7	26.6	1.056	325
Resh-3	08.10.97	15.0	17.1	0.784	96
AVERAGE		34.1	18.7	0.9	157
STD. ERROR		9.7	4.2	0.1	84
UPSTREAM:					
Ch-R-1	06.06.97	1.8	17.0	<0.1	39
Ch-R-2	06.06.97	2.1	18.0	1.0	74
Ch-R-3	06.06.97	4.4	18.0	0.5	37
Ch-R-4	10.07.97	0.9	9.3	0.10	16
Ch-R-5	10.07.97	1.7	10.6	0.26	65
Ch-R-6	10.07.97	0	10.1	0.31	20
Ch-R-7	27.08.97	0	10.9	0.45	28
Ch-R-8	27.08.97	0	8.0	0.06	30
Ch-R-9	27.08.97	not available	9.5	1.80	38
Ch-R-10	19.09.97	2.2	7.6	0.29	16
Ch-R-11	19.09.97	3.4	13.5	0.55	36
Ch-R-12	19.09.97	3.0	8.5	0.14	24
AVERAGE		2.0	11.8	0.5	35
STD. ERROR		1.0	2	0.1	5
DOWNSTREAM:					
Ch-DS-1	06.06.97	830	30	0.5	99
Ch-DS-2	06.06.97	820	28	0.2	62
Ch-DS-3	06.06.97	790	26	0.2	54
Ch-DS-4	10.07.97	670	48.1	0.386	109
Ch-DS-5	10.07.97	850	54.5	0.679	130
Ch-DS-6	10.07.97	833	56.2	0.715	161
Ch-DS-7	22.08.97	628	26	0.655	92
Ch-DS-8	22.08.97	900	38	0.552	117
Ch-DS-9	22.08.97	1070	42.8	1.061	189
Ch-DS-10	26.09.97	1193	23.2	0.686	42
Ch-DS-11	26.09.97	1233	24.2	0.644	36
Ch-DS-12	26.09.97	1245	24.4	0.415	35
AVERAGE		922	35.1	0.6	94
STD. ERROR		102	6.4	0.1	24
TRIBUTARY:					
Ch-Pa-1	10.07.97	6000	91		58
Ch-Pa-2	10.07.97	6500	92		144
Ch-Pa-3	10.07.97	6300	82		84
Ch-Pa-4	08.10.97	5940	90		96
Ch-Pa-5	08.10.97	5920	87		87
Ch-Pa-6	08.10.97	6060	95		164
AVERAGE		6120	90	NA	106
STD. ERROR		147	1	NA	10

WATER CHEMISTRY - DATA

parameter	UPSTREAM SITE						RESHOTKA	DOWNSTREAM SITE						PAHOTKA
	06.06.97	10.07.97	27.08.97	18.09.97	average	std. error	08.10.97	06.06.97	10.07.97	27.08.97	26.09.97	average	std. error	10.07.97
pH	8.16	7.7	7.49	7.48	7.71	0.15913	7.16	8.38	7.09	7.4	7.14	7.50	0.30	7.63
Pt-Co	32.5	31.6	36	30.5	32.7	1.19	73	82	76.9	98	110	91.7	7.57	402.5
O_2	11.35	9.28	10.50	11.92	10.76	0.574	12.81	10.51	9.81	10.62	12.17	10.78	0.50	N
Na	5.8	6.7	5.35	5.75	5.90	0.285	5	24.3	23.3	16.6	23.2	21.9	1.77	127
K	0.8	12.8	1.1	1.1	4.0	3.0	1	1.25	2.5	1.5	1.5	1.7	0.28	3.7
Ca	19.04	27.81	29.06	32.36	27.07	2.843	26.55	41.08	38.65	39.83	39.08	39.66	0.53	48.59
Mg	9.12	10.82	8.21	8.63	9.20	0.5727	6.26	8.21	6.99	6.69	8.82	7.68	0.50	21.88
Fe(II)	N	N	0.001	N	0.001		N	N	N	N	N	N	N	0.003
Fe(tot.)	0.15	0.125	0.124	0.118	0.129	0.0071	0.374	0.166	0.202	0.13	0.157	0.164	0.01	0.16
HCO3	57.34	95.16	70.76	77.47	75.18	7.865	73.2	61	85.4	70.76	63.44	70.15	5.49	112.24
SO4	54.72	52.8	48.96	47.04	50.88	1.753	41.28	115.2	103.68	77.76		98.88	9.59	495.3
Cl	64.66	11.83	9.36	10.37	24.06	13.54	8.04	21.58	15.47	19.63	25.09	20.44	2.01	38.35
NH4	0.37	0.115	0.34	0.155	0.245	0.0643	0.3	0.33	0.15	0.33	0.3	0.28	0.04	0.255
NO2	0.008	0.004	0.007	0.004	0.006	0.0010	0.005	0.031	0.04	0.007	0.047	0.031	0.01	0.114
NO3	0.06	0.06	0.075	0.07	0.07	0.0037	0.16	0.56	0.925	0.075	1.03	0.648	0.22	1.25
N(tot.)	0.843	0.739	0.807	0.724	0.778	0.0281	0.915	1.416	1.59	0.807	1.702	1.379	0.20	2.289
P(tot.)	0.047	0.045	0.025	0.022	0.035	0.0065	0.03	0.116	0.274	0.025	0.35	0.191	0.07	0.689
P(min.)	0.002	0.005	0.001	N	0.003	0.001	0.013	0.034	0.139	0.001	0.24	0.10	0.05	0.288
COD	34.44	33.71	23.67	20.37	28.05	3.548	26.02	34.12	26.19	23.67	27.11	27.77	2.24	42.92
BOD5	*3.79	3.09	1.61	1.87	2.19	0.395	0.65	*3.27	2.33	1.61	*1.53	1.97	0.25	3.09
susp. part.	26	7.7	2.05	11.85	11.9	5.11	2.6	11.55	13.7	2.05	2.1	7.35	3.08	12.1
Temp.	22.7	20.4	17.9	13.9	18.7	1.88	6.2	21.4	17.9	18.3	10.3	17.0	2.36	19.2
conductiv.	0.213	0.305	0.265	0.283	0.267	0.0196	0.232	0.450	0.445	0.371	0.427	0.423	0.0181	1.7
v	0.1	0.12	0.1	0.1	0.11	0.005	N	0.5	0.5	0.4	0.4	0.45	0.03	N
Q	0.2	0.2	0.2	0.2	0.2	0	N	6.5	7	4.5	2.8	5.2	0.97	N
Eh	1.00	1.02	1.03	1.03	1.02	0.00583	1.04	1.00	1.04	1.03	1.04	1.03	0.01	N

parameters in mg/L except pH, Pt-Co units, temperature (deg C), conductivity (µS/cm), velocity (m/s), discharge (m^3/s), Eh (V)

(N= data not available)

SEDIMENT - TOTAL METALS EXTRACTION - DATA															
UPSTREAM:															
sample ID	dry wt. (g)	µg Cr/g	µg Cr/g	µg Mn/g	µg Mn/g	µg Zn/g	µg Zn/g	µg Cu/g	µg Cu/g	µg Cd/g	µg Cd/g	µg Ni/g	µg Ni/g	µg Pb/g	µg Pb/g
R-1A	0.5576	108	106	2401	2408	890	890	973	979	11	11	108	110	136	138
R-1B	0.4958	105		2414		891		985		12		112		139	
R-2A	0.5251	88	94	5527	5236	1143	1078	940	900	13	13	117	113	152	145
R-2B	0.5562	101		4946		1014		860		12		109		138	
R-3A	0.5425	90	87	2879	2639	804	739	557	514	8	8	93.9	87	90	82
R-3B	0.5933	84		2399		674		472		7		80.5		74	
R-4A	0.4969	107	108	4484	4605	867	879	684	697	10	11	106.5	108	121	123
R-4B	0.5774	109		4726		890		710		11		109.1		125	
R-5A	0.4853	120	112	3231	2998	1191	1097	976	895	15	13	121.0	113	193	178
R-5B	0.4729	104		2766		1002		815		12		104.6		163	
R-6A	0.4975	107	108	3906	3813	780	768	657	647	10	10	106.2	106	117	117
R-6B	0.5633	110		3721		756		637		9		104.9		117	
R-7A	0.5658	92	95	2229	2291	550	543	396	398	6	6	94.0	94	71	71
R-7B	0.5893	98		2354		536		400		6		94.7		71	
R-8A	0.5746	99	98	2443	2453	475	475	285	286	5	5	89.8	90	57	59
R-8B	0.5014	98		2463		475		286		6		90.7		60	
R-9A	0.5664	95	95	2007	2027	408	421	268	276	5	5	90.0	91	53	54
R-9B	0.5646	96		2047		434		284		5		91.6		55	
DOWNSTREAM:															
DS-1A	0.6121	2553	2508	1720	1711	621	617	588	586	6	6	279.6	281	96	96
DS-1B	0.5292	2462		1703		614		584		6		281.9		96	
DS-2A	0.4822	3013	2899	1896	1834	742	699	678	651	7	6	307.2	294	114	112
DS-2B	0.5656	2784		1771		656		624		6		279.8		110	
DS-3A	0.4803	1874	1889	1518	1530	537	545	546	553	5	5	180.5	183	90	92
DS-3B	0.5188	1904		1542		553		561		5		184.7		94	
DS-4A	0.5562	2595	2544	1624	1631	701	696	739	736	6	6	335.3	336	110	112
DS-4B	0.5646	2494		1638		691		733		6		336.3		115	
DS-5A	0.5021	1882	1784	1382	1350	540	528	564	551	5	5	227.8	221	98	95
DS-5B	0.5834	1685		1318		516		538		5		215.1		93	
DS-6A	0.5941	2769	3005	1301	1424	638	694	675	732	6	6	305.7	329	111	120
DS-6B	0.5284	3242		1546		749		789		7		352.8		129	
TRIBUTARY:															
Pa-1A	0.5010	7884	6967	1910	1784	381	346	288	267	4	4	444.8	398	78	72
Pa-1B	0.5843	6050		1658		311		246		4		350.7		67	
Pa-2A	0.5208	4964	4918	1576	1843	355	357	311	309	4	4	348.8	347	79	80
Pa-2B	0.5276	4871		2110		358		307		4		346.2		81	
Pa-3A	0.5119	8185	9026	2487	2834	441	479	335	373	5	6	482.3	494	70	74
Pa-3B	0.5888	9868		3181		516		410		6		506.5		78	
UPSTREAM averages:		101		3163		766		621		9		101.31		107.4	
REF. standard error		3		377		82		90		1		3.50		14.4	
DOWNSTREAM averages:		2438		1580		630		635		6		273.90		104.6	
downstream standard error		207		74		32		35		0		24.77		4.7	
TRIBUTARY averages:		6970		2154		394		316		5		413.23		75.5	
TRIBUTARY standard error		1186		340		43		31		0		43.12		2.4	

APPENDIX III

SEDIMENT - BIOAVAILABLE METALS ($CaCl_2$ extraction) - DATA

UPSTREAM:								
sample ID	extract pH	dry wt. (g)	Cr(µg/g)	Cr(µg/g)	Cu(µg/g)	Cu(µg/g)	Fe (µg/g)	Fe (µg/g)
R1-I	7.14	4.6420	0.000		0.365		0.27	
R1-II	7.20	4.5453	0.000	0.00	0.450	0.408	0.50	0.38
R2-I	7.03	4.7804	0.000		0.179		0.40	
R2-II	7.04	4.8120	0.000	0.00	0.158	0.168	0.21	0.30
R3-I	6.74	4.7710	0.005		0.088		0.30	
R3-II	6.73	4.8771	0.001	0.00	0.082	0.085	0.38	0.34
R4-I	6.96	4.8759	0.001		0.155		0.68	
R4-II	6.99	4.8892	0.000	0.00	0.110	0.133	0.29	0.48
R5-I	7.26	4.8483	0.003		0.102		0.38	
R5-II	7.22	4.8661	0.003	0.00	0.103	0.102	0.40	0.39
R6-I	7.02	4.5528	0.000		0.105		0.15	
R6-II	7.05	4.5479	0.000	0.00	0.110	0.108	0.44	0.30
R7-I	6.93	4.9022	0.012		0.105		0.22	
R7-II	7.03	5.0262	0.004	0.01	0.080	0.092	0.39	0.31
R8-I	6.77	4.8238	0.002		0.074		0.37	
R8-II	6.88	4.8554	0.002	0.00	0.060	0.067	0.29	0.33
DOWNSTREAM:								
DS1A-I	7.15	4.8744	0.014		0.099		0.15	
DS1A-II	7.16	4.9276	0.016	0.015	0.099	0.099	0.38	0.26
DS2A-I	7.23	4.9465	0.016		0.099		0.11	
DS2A-II	7.31	4.9223	0.016	0.016	0.082	0.091	0.16	0.14
DS3A-I	7.16	4.8807	0.024		0.106		0.31	
DS3A-II	7.10	4.8476	0.019	0.021	0.128	0.117	0.80	0.56
DS4B-I	7.20	4.8094	0.009		0.105		0.06	
DS4B-II	7.22	4.8137	0.015	0.012	0.089	0.097	0.45	0.25
DS5A-I	7.29	4.8994	0.010		0.108		0.16	
DS5A-II	7.24	4.8550	0.009	0.010	0.116	0.112	0.56	0.36
DS6A-I	7.13	4.8887	0.016		0.094		0.41	
DS6A-II	7.22	4.8644	0.009	0.013	0.071	0.083	0.48	0.45
TRIBUTARY:								
Pa1-I	7.67	4.8646	0.184		0.105		1.33	
Pa1-II	7.71	4.9047	0.143	0.163	0.083	0.094	0.45	0.89
Pa2-I	7.58	4.8287	0.079		0.087		0.30	
Pa2-II	7.61	4.9035	0.066	0.072	0.090	0.088	0.55	0.43
Pa3-I	7.70	4.8775	0.099		0.112		0.49	
Pa3-II	7.69	4.8740	0.093	0.096	0.097	0.105	0.40	0.45
		AVERAGE Cr	STD ERROR		AVERAGE C	STD ERROR		
UPSTREAM		0.002	0.001		0.145	0.03904		
DOWNSTREAM		0.015	0.002		0.100	0.00526		
TRIBUTARY		0.111	0.027		0.096	0.00478		
		AVERAGE Fe	STD ERROR					
		0.354	0.022					
		0.336	0.061					
		0.586	0.151					

APPENDIX IV

DETRITUS EXTRACTIONS - DATA					
UPSTREAM:					
SAMPLE ID	DRY WT (mg)	corr.Cr(μg/L)	μg Cr/g	μg Cu/L	μg Cu/g
R2	2.38	70	60	1232	1061
R3	2.51	118	96	1639	1339
R4	2.55	136	109	1872	1505
R6	2.26	117	106	1329	1206
R7	2.31	180	160	1555	1380
R8	2.30	118	105	1750	1560
R10	2.34	90	79	1796	1573
R11	2.47	88	73	1488	1235
R12	2.33	157	138	1622	1427
R14	2.27	121	109	1658	1497
R15	2.33	99	87	1523	1340
AVERAGE:			102		1375
STANDARD ERROR:			9		48
DOWNSTREAM:					
DS1	2.43	19200	16198	1161	979.4
DS3	2.51	26900	21970	1254	1024
DS4	2.31	23200	20589	1184	1051
DS5	2.33	20900	18388	1123	988.0
DS7	2.63	17300	13485	1042	812.2
DS8	2.23	18700	17191	1094	1006
DS9	2.31	18400	16329	1136	1008
DS11	2.67	18100	13897	1247	957.4
DS12	2.73	21900	16445	1297	973.9
DS13	2.32	19000	16789	1153	1019
DS15	2.61	18200	14295	1172	920.5
AVERAGE:			16870		976.3
STANDARD ERROR:			798		19.6
BRS-1	2.32	85	75.1078	107	94.547
BRS-2	2.33	89	78.3047	103	90.622
BRS-3	2.25	86	78.3556	113	102.956
BRS (NIST 2704)					

BENTHIC MACROINVERTEBRATE COMMUNITY, Chusovaya River

upstream	6.06.97				10.07.97						27.08.97						19.09.97					
taxa	Mud 0,1 m.sq.		Gravel 0,1 m.sq		AS (glass)		AS (glass)		AS (ceramic)		Sand(0,3 m.sq)		Gravel(0,3 m.sq)		Qual (10 stones)		AS (ceramic)		AS (ceramic)		AS (glass)	
Aranei																	1	0,005				
Ablabesmyia lentiginosa													1	0,005			1	0,005	1	0,005	1	0,005
Ablabesmyia monilis					3	0,005	6	0,01	22	0,045			1	0,005								
Amesoda sp.	1	0,005									1	0,03	16	0,115								
Ancylus fluviatilis															1	0,01						
Anisus sp.											1	0,005			1	0,005						
Athripsodes annulicornis									5	0,02					2	0,01						
Athripsodes annulicornis (pup)									1	0,005												
Athripsodes cinereus													2	0,005								
Baetis sp.					1	0,005	1	0,005									1	0,005				
Bezzia sp.													1	0,005								
Caenis undosa							1	0,005														
Caenis horaria	1	0,005					1	0,005	2	0,005	1	0,005	22	0,02	2	0,005	22	0,015	11	0,01	4	0,01
Caenis moesta					2	0,01	1	0,005	1	0,005												
Calanoida							5	0,005	6	0,01												
Ceratopogon sp.											2	0,005	2	0,005								
Chironominae (pup)									4	0,015												
Chironomus dorsalis			1	0,005																		
Chironomus sp.					1	0,005																
Chironomus sp.(pup)					1	0,01																
Chrysops sp.													1	0,035								
Cladotanytarsus sp.					1	0,005					1	0,005										
Clinotanytarsus nervosus											2	0,005										
Corynoneura celeripes							1	0,005														
Cricotopus sp.															48	0,07						
Cricotopus sp.(pup)															8	0,015						
Cryptochironomus conjungens					7	0,01	7	0,01	3	0,005			3	0,01			3	0,005				
Cryptochironomus defectus	1	0,005																				
Cryptochironomus sp.													1	0,005	4	0,005						
Cryptochironomus vulneratus													17	0,015								
Cyrnus flavidus					15	0,065	9	0,03	2	0,01												
Cyrnus sp.(pup)					1	0,01																
Diamesa sp.			3	0,005					3	0,005					20	0,025						
Endochironomus albipennis											21	0,03	8	0,01	24	0,035	30	0,045	15	0,035	5	0,015
Endochironomus donatoris			1	0,005																		
Endochironomus impar													1	0,005								
Endochironomus sp.					23	0,03	25	0,025	76	0,175												
Ephemera vulgata													4	0,015	2	0,015						
Erpobdella octoculata							7	0,61	1	0,005	1	0,115			2	0,025			3	0,045	3	0,05
Erpobdella sp.					3	0,005																
Erythromma najas	1	0,045																				
Euglesa sp.	7	0,08									6	0,05	67	0,35								
Eucyclopinae																			1	0,005		
Eukiefferiella sp.							1	0,005					6	0,005	40	0,06						

upstream	6.06.97				10.07.97						27.08.97						19.09.97					
taxa	Mud 0,1 m.sq.		Gravel 0,1 m.sq		AS (glass)		AS (glass)		AS (ceramic)		Sand(0,3 m.sq)		Gravel(0,3 m.sq)		Qual (10 stones)		AS (ceramic)		AS (ceramic)		AS (glass)	
fam. Corixidae											1	0,005										
Glossiphonia complanata					5	0,135	3	0,01	1	0,005	1	0,045	1	0,02								
Glyptotendipes glaucus					26	0,035	30	0,035	69	0,16			1	0,005			15	0,025	11	0,025	4	0,01
Glyptotendipes sp.					2	0,005					1	0,005			16	0,025						
Gordius sp.									2	0,095												
Halesus radiatus	1	0,145																				
Haliplus ruficollis (imago)									1	0,005												
Helobdella stagnalis					1	0,005	1	0,005			1	0,01	1	0,01	1	0,005						
Helobia sp.											1	0,005										
Helophorus sp.(imago)					1	0,005																
Hydaticus sp.(larvae)					6	0,025	5	0,015	6	0,015												
Hydra							1	0,005							4	0,005						
Hydracarina							1	0,005														
Hydropsyche pellucidula			2	0,1																		
Hydropsyche sp.(pup)			1	0,02																		
Ilybius sp.(larvae)					20	0,11	7	0,035	2	0,005							2	0,015	1	0,005	1	0,015
Limnochironomus nervosus									2	0,005			1	0,005								
Limnochironomus sp.					9	0,015	2	0,005														
Limnodrilus sp.											7	0,03	8	0,015								
Limnophora sp.																	2	0,005				
Lymnaea glutinosa															1	0,12						
Lymnaea intermedia									3	0,1												
Lymnaea ovata	1	0,595							3	0,015												
Lymnaea patula											1	0,085					1	0,035				
Mallochohelea sp.													1	0,005								
Micronecta sp.													1	0,005								
Micropsectra sp.													7	0,005								
Microtendipes chloris							15	0,02					3	0,005								
Microtendipes sp.					11	0,015			1	0,005			1	0,005								
Nematoda															1	0,005						
Nepa cinerea	1	0,27																				
Neureclipsis bimaculata			1	0,03									1	0,005			1	0,005				
Orthocladius sp.													1	0,005	8	0,01						
Oxyethra sp.					1	0,005																
Paraleptophlebia submarginata																	1	0,005	5	0,015		
Paratanytarsus sp.			1	0,005																		
Peloscolex ferox													21	0,03								
Pentapedilum exectum											9	0,01	12	0,015								
Physa fontinalis															1	0,005						
Polycentropus flavomaculatus							1	0,005					4	0,01			8	0,015	9	0,015	2	0,01
Polypedilum convictum			1	0,005							3	0,005	5	0,01								
Polypedilum nubeculosum															8	0,01						
Polypedilum sp.					6	0,01	2	0,005	10	0,015			3	0,01								
Polypedilum sp.(pup)					2	0,01																
Potamonectes sp.(imago)									1	0,005												
Prodiamesa sp.															4	0,005						

upstream	6.06.97				10.07.97						27.08.97						19.09.97					
taxa	Mud 0,1 m.sq.		Gravel 0,1 m.sq		AS (glass)		AS (glass)		AS (ceramic)		Sand(0,3 m.sq)		Gravel(0,3 m.sq)		Qual (10 stones)		AS (ceramic)		AS (ceramic)		AS (glass)	
Psectrocladius sp.					2	0,005	5	0,005	7	0,01	3	0,005	5	0,015	56	0,08			1	0,005		
Rheotanytarsus sp.													1	0,005								
Sialis sp.	4	0,115									4	0,16										
Sida crystallina					13	0,01	13	0,015	72	0,1					80	0,12	67	0,03	44	0,04	40	0,05
Simulium sp.																	1	0,005				
Sphaerium corneum													3	0,85								
Sphaerium sp.					1	0,005							4	0,145								
Stylaria lacustris					114	0,04	89	0,04	89	0,075					4	0,005	1	0,005			1	0,005
Tabanus sp.													1	0,015								
Tanypus sp.											3	0,01										
Tanytarsini(pup)					1	0,005																
Tanytarsus sp.					11	0,015	17	0,01	5	0,005			5	0,005								
Thienemanniella sp.															4	0,005						
Tribelos sp.					1	0,005																
Valvata cristata															3	0,01						
Valvata depressa																			1	0,01		
Valvata sp.							2	0,01														
Orthocladiinae(pup)							6	0,01	1	0,005			4	0,01								
fam. Enchytraeidae					1	0,005																
fam. Helodidae (imago)			1	0,01																		

BENTHIC MACROINVERTEBRATE COMMUNITY, Chusovaya River

downstream	10.07.97						28.08.97						26.09.97					
taxa	AS (glass)		AS (glass)		AS (ceramic)		AS(ceramic)		AS(glass)		AS(glass)		AS (ceramic)		AS (glass)		AS (glass)	
Ablabesmyia lentiginosa	144	0,215	310	0,385	460	0,71	189	0,2	183	0,145	198	0,16	43	0,015	44	0,015	32	0,02
Ablabesmyia monilis	310	0,43	196	0,245	332	0,51	21	0,02	9	0,01	27	0,02	2	0,005			4	0,005
Ablabesmyia sp.(pup)	26	0,04	36	0,07	56	0,12	15	0,015	3	0,01	6	0,005						
Amesoda sp.			2	0,01														
Atherix sp.	9	0,035	14	0,165	23	0,26	11	0,81	1	0,035	5	0,34	2	0,105	3	0,135		
Athripsodes annulicornis	1	0,005			2	0,005												
Athripsodes cinereus											2	0,005						
Baetis vernus	2	0,005	2	0,005	1	0,005	3	0,01							1	0,005		
Caenis horaria							4	0,01	2	0,005								
Callicorixa praeusta																	3	0,065
Clinotanypus nervosus							9	0,01										
Cricotopus sp.	26	0,01	130	0,05	280	0,075									2	0,005		
Cryptochironomus conjungens			6	0,005					3	0,005	21	0,015						
Cryptochironomus sp.	2	0,005	6	0,005			3	0,005										
Culicoides sp.							2	0,005										
Cyrnus flavidus							1	0,005										
Diamesa sp.	12	0,005	18	0,01	10	0,005												
Endochironomus albipennis							3	0,005	3	0,01	12	0,01						
Endochironomus tendens					2	0,005												
Ephemerella ignita			1	0,01														
Erpobdella octoculata							1	0,045	1	0,005	2	0,005					1	0,005
Eukiefferiella sp.	62	0,02	66	0,025	64	0,02			3	0,005			4	0,005	12	0,005	18	0,01
fam. Polycentropodidae (pup)							1	0,01										
Glyptotendipes glaucus							15	0,015	21	0,025	36	0,03						
Glyptotendipes sp.					2	0,005												
Guttipelopia sp.	4	0,005																
Helobdella stagnalis							1	0,01					1	0,005			1	0,005
Hexatoma sp.									1	0,03								
Hydra																	1	0,005
Hydropsyche angustipennis													5	0,115			1	0,005
Hydropsyche nevae	1	0,005	1	0,005											1	0,015		
Hydropsyche contubernalis	43	0,83	61	0,68	52	0,655	240	0,735	140	0,55	256	1,105	22	0,31	58	0,65	55	0,61
Hydropsyche contubernalis(pup)																		
Hydropsyche pellucidula	11	0,13	32	0,35	29	0,28	29	0,615	26	0,18	42	0,42	2	0,09	1	0,055	1	0,06
Hydropsyche sp.(pup)											2	0,03						
Leuctra sp.															1	0,005		
Leptoceridae indet.															1	0,005		
Limnochironomus sp.			2	0,005	2	0,005			3	0,005	6	0,005						

Taxa	10.07.97						28.08.97						26.09.97					
	AS (glass)		AS (glass)		AS (ceramic)		AS(ceramic)		AS(glass)		AS(glass)		AS (ceramic)		AS (glass)		AS (glass)	
Limnochironomus nervosus													1	0,005				
Musculium hungaricum									1	0,07								
Neureclipsis bimaculata	1	0,03	3	0,08	5	0,08	33	0,125	23	0,095	49	0,215			8	0,01	7	0,03
Neureclipsis bimaculata(pup)	2	0,04	1	0,01														
Mermitidae													1	0,005			2	0,005
Orthocladius sp.											6	0,005			1	0,005	3	0,005
Paracorixa concinna																	2	0,04
Pentapedilum sp.			2	0,005	4	0,005												
Platycnemis pennipes											1	0,025						
Polycentropus flavomaculatus					1	0,01	1	0,005	3	0,005	3	0,01					1	0,005
Prodiamesa olivacea							2	0,005										
Prodiamesa olivacea(pup)									6	0,01								
Prodiamesa sp.													1	0,005	2	0,005		
Psectrocladius sp.	106	0,035	64	0,025	80	0,025	15	0,015	3	0,005	12	0,01	1	0,005	2	0,005	1	0,005
Sialis sp.	3	0,01	3	0,01	2	0,005	2	0,04										
Sigara assimilis													1	0,015				
Simulium sp.	6	0,005	5	0,01	1	0,005	12	0,015			6	0,005						
Simulium sp.(pup)											3	0,005						
Tabanus sp.	1	0,045											1	0,275				
Tanytarsus sp.	2	0,005	2	0,005	4	0,005												
•/•••.Orthocladiinae(pup)	6	0,005	8	0,01	10	0,01			6	0,005	18	0,015						

APPENDIX VI

BIOACCUMULATION DATA - *Hydropsyche pellucidula*

	REFERENCE (Reshotka)		8.10.97				DOWNSTREAM (Chusovaya)		18.10.97		
Hp-R	dry wt. (mg)	Cr (µg/L)	Cr (µg/g)	Cu (µg/L)	Cu (µg/g)	Hp-DS	dry wt. (mg)	Cr (µg/L)	Cr (µg/g)	Cu (µg/L)	Cu (µg/g)
1	8.874	7.3	1.6	83	18.7	1	14.166	407	57.5	263	37.1
2	12.258	7.9	1.3	142	23.2	2	14.852	504	67.9	378	50.9
3	10.292	6.7	1.3	99	19.2	3	16.662	457	54.9	332	39.9
4	11.746	7.4	1.3	145	24.7	4	15.502	334	43.1	364	47.0
5	10.426	9.5	1.8	146	28.0	5	20.886	638	61.1	549	52.6
6	14.096	7.4	1.0	146	20.7	6	17.010	335	39.4	283	33.3
7	19.056	7.3	0.8	156	16.4	7	14.566	445	61.1	424	58.2
8	9.316	10.2	2.2	121	26.0	8	17.784	412	46.3	251	28.2
9	11.686	6.3	1.1	99	16.9	9	12.328	416	67.5	308	50.0
10	12.726	7.2	1.1	126	19.8	10	14.670	355	48.4	189	25.8
11	9.754	6.7	1.4	91	18.7	11	7.132	228	63.9	131	36.7
12	11.084	7.6	1.4	123	22.2	12	18.898	480	50.8	464	49.1
13	10.700	6.8	1.3	100	18.7	13	8.600	265	61.6	211	49.1
14	8.732	7.9	1.8	98	22.4	14	15.014	326	43.4	382	50.9
15	10.398	6.1	1.2	80	15.4	15*	3.586	170	94.8	199	111.0
16	11.134	6.0	1.1	89	16.0	16*	1.188	29	48.8	58	97.6
17	5.594	4.6	1.6	38	13.6	17*	1.556	42	54.0	87	111.8
18	10.500	8.3	1.6	77	14.7	18*	1.638	49	59.8	67	81.8
19	6.145	4.0	1.3	45	14.6	19*	1.430	34	47.6	39	54.5
20	8.722	10.5	2.4	91	20.9						
		average:	1.4		19.5			average:	54.8		56.1
		std. error	0.1		0.9			std. error	2.2		5.9

* fourth instars

APPENDIX VII

PHYSICAL ABNORMALITIES - *Hydropsyche pellucidula*

downstream			
	length (mm)	gill damage	anal papilla damage
H.p.1	16	2	1ap totally black, 3 white
H.p.2	18	3	1 tip dark, 3 white
H.p.3	16	3	4 clear
H.p.4	18	1	1 tip dark, 3 clear
H.p.5	19	2	3 clear, 1 gray
H.p.6	15	2	3 clear, 1 darkened tip
H.p.7	16	2	3 half-dark (4th not seen)
H.p.8	18	2	2 clear, 2 graying
H.p.9	15	1	4 clear
H.p.10	15	1	1 clear, 2 tips brownspots,1 tip dark
H.p.11	14	2	3 clear, 1 with some dark dots
H.p.12	17	3	2 clear, 1 with dark ring, 1 graying
H.p.13	14	0	3 clear, 1 gray
H.p.14	17	2	2 clear/white, 2 with very black tips
H.p.15	10	1	4 very clear
H.p.16	8	2	NOT SEEN (larva too small)
average		1.8	80% affected by damage
std. error		0.2	
reference			
H.p.1	14	1	1 half dark, 1 very dark
H.p.2	15	1	1 half dark
H.p.3	16	0	all light
H.p.4	14	3	all light
H.p.5	15	1	all white
H.p.6	18	2	all light
H.p.7	19	1	all light
H.p.8	15	1	all light
H.p.9	15	0	all light
H.p.10	17	0	all light
H.p.11	15	0	all light
H.p.12	15	1	all light
H.p.13	16	3	all light
H.p.14	15	0	all light
H.p.15	14	0	1-dark band middle of papilla
H.p.16	14	0	all light
H.p.17	13	0	all white
H.p.18	17	2	all light
H.p.19	14	1	all light
H.p.20	15	1	all light
average		0.9	20% affected by damage
std. error		0.2	

98.