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On the Criteria for the Assessment of the State of Polluted Freshwater Ecosystems with an example of the Small River on the Eastern Edge of Europe (Perm Krai, Russia)

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ABSTRACT

The use of abiotic ecological indicators in environmental monitoring does not always reliably reflect the level of anthropogenic impact on aquatic ecosystem. Our study aims to estimate possible differences in abiotic and biotic index-based assessments of the impact of industrial pollution on water quality in the small boreal river located at the eastern edge of Europe (Perm Krai, Russia). We analyzed the contamination of an 13-km long river by major organic and inorganic ions, trace elements, and thermal pollution. To evaluate the toxicity of bottom sediments, threshold effect concentration (TEC), probable effect concentration (PEC), and potential effect concentration quotient (mPECQ) were calculated from the concentration of heavy metals. Also, benthic macroinvertebrates were sampled from headwaters to the mouth of the river. We found the presence of the members of the EPT taxa at the sites with a high concentration of heavy metals, which can be explained by the tolerance of local invertebrates to specific pollutants. Apart from that, our results show that in the middle reach of the river, toxic effect of a nitrite-ion high concentration contradicts the presence of gill breathing crustaceans sensitive to this type of pollution. This can be explained by the high concentration of chlorides at this site, which leads to reduction of nitrite toxicity. Further work should be focused on developing abiotic indices that account for the sensitivity of local invertebrates to specific pollutants, while comprehensive pollution assessment should include antagonistic or synergistic interactions of various pollutants.

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INTRODUCTION

The water quality of rivers and lakes around the world is decreasing due to climate change and the negative impact of human activity such as discharge of sewage and drainage waters from the enterprises of various industries, and also effluents associated with agriculture and mining activities (Opekunov et al., 2020; Zubarev, 2020; Baryshev, 2021; Fetisova, 2021). In many countries, biological indicators integrated into environmental assessment are using to monitor and manage freshwater resources (Savitskaya, 2017). Modern studies show that an integrated approach should be used in the environmental assessment of water quality, which includes the analysis of both abiotic and biological indicators (Nawrot et al., 2021; Wang, 2023). Aquatic

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ecosystems' assessment derived from the results of geochemical and ecotoxicological studies of surface waters and bottom sediments does not always reliably reflect the level of anthropogenic impact on freshwaters. Unlike physico-chemical assessment of water quality, bioindication can be a more useful tool for water quality assessment because it does not demand any expensive equipment and is characterized by low research costs (Babko et al, 2019; Girotti et al., 2020). Various ecological groups of hydrobionts can be used as bioindicators – bacterial communities, phyto- and zooplankton, periphyton, macrozoobenthos, as well as ichthyofauna (Kadim & Risjani, 2022; Reid et al., 2024).

Previous studies have shown that the pollution of the rivers, for example the Wei River (China), the Furnia River (Spain), the Vyatka River (Russia), the Vychegda River (Russia), leads to negative changes in the diversity and abundance of macrozoobenthos, which is often used as bioindicator to assess ecosystem quality (Kochurova, 2020; Baturina et al., 2021; Zhang et al., 2021; Gutiérrez et al., 2022). The biotic indices usually used for water quality assessment, are based on the data on invertebrates determined up to the family level (Baryshev, 2021; Golovatyuk, Mikhailov, 2021). At the same time, several indices are most often referred to at once, compensating for each other's shortcomings. As biotic indices based on the structural characteristics of macrozoobenthos are the most convenient to use, they are the most popular ones (Andrianova, 2015; Musonge et al., 2020).

According to the state report, the total volume of wastewaters in Russia in 2020 amounted to 34,232.3 million m^3, and only 8% of wastewaters were treated (On the state…, 2021). Perm Krai annually discharges 69.9 million m^3 of polluted untreated wastewater into the natural environment. Over the past decades, the water quality in most rivers of Perm Krai does not meet fisheries standards, which is confirmed by the results of hydrochemical observations (On the condition…, 2021; Toropov, 2019).

Solikamsk-Berezniki agglomeration is located in the northern part of Perm Krai, where large industrial enterprises of non-ferrous metallurgy, chemical, mining industries are located. As a result of industrial activity, wastewater is discharged into small tributaries of the Kama River. The water and bottom sediments of these rivers, as well as the Kama River near the Solikamsk-Berezniki industrial zone are characterized by high concentrations of major chemical macro-components and trace elements (Alexander et al., 2007; Menshikova, 2016; Belkin, 2018; Miroshnichenko, 2019; Lepikhin et al., 2020; Ushakova et al., 2020; Khayrulina et al., 2022). The water quality is often categorized as the 3rd class or "very polluted" (On the condition…, 2021). The effect of industrial wastewater discharges on the composition of macrozoobenthos in small rivers of both the Solikamsk-Berezniki agglomeration and the boreal forest zone of the eastern edge of Europe has not been studied. At the same time, small rivers are the main source of pollution for larger waterbodies (Toropov, 2019).

The aim of the study is to assess the pollution of the small boreal river in Perm Krai, affected by wastewater discharge, by referring to macrozoobenthos data and abiotic indicators. Also, our study examines differences in the water quality assessment arisen from the application of various abiotic and biotic indices.

MATERIALS AND METHODS

Study Area

The Tolych River is the eastern tributary of the Kama River, which is located in Perm Krai, Russia (59◦26′ N and 56◦44′ E). The Tolych River has a length of 13 km and a catchment area of 36.1 km^2. The main tributary of the river is the 6-km long Zatolych River which confluences with the Tolych River 3.8 km from its mouth. According to the data provided by the State Water Registry, the average annual water discharge of the Tolych River at 1.5 km from its mouth is 0.33 m^3/sec. The Tolych River flows through the industrial area of Berezniki City, where large industrial enterprises of non-ferrous metallurgy, chemical and energy industries are located and discharge wastewater in the river catchment. In 2021, the annual volume of the industrial wastewater sewage directly released into the Tolych River comprised 52.9 million m^3, with its 90% being untreated. Wastewater disposal in the Tolych River basin is uneven from upstream to downstream. Therefore, the flow of the Tolych River is affected by the fluctuations associated with sewage discharges. The natural landscape of boreal forest with minimal anthropogenic impact is presented only at the headwaters of the river catchment.

Sampling and measurements

Water, bottom sediments, and macroinvertebrate samples were collected in August 2021 at 6 sampling stations located upstream and downstream from the industrial sewage discharges (Fig. 1). All samples were taken at one day. The air temperature during the sampling was 25°C.

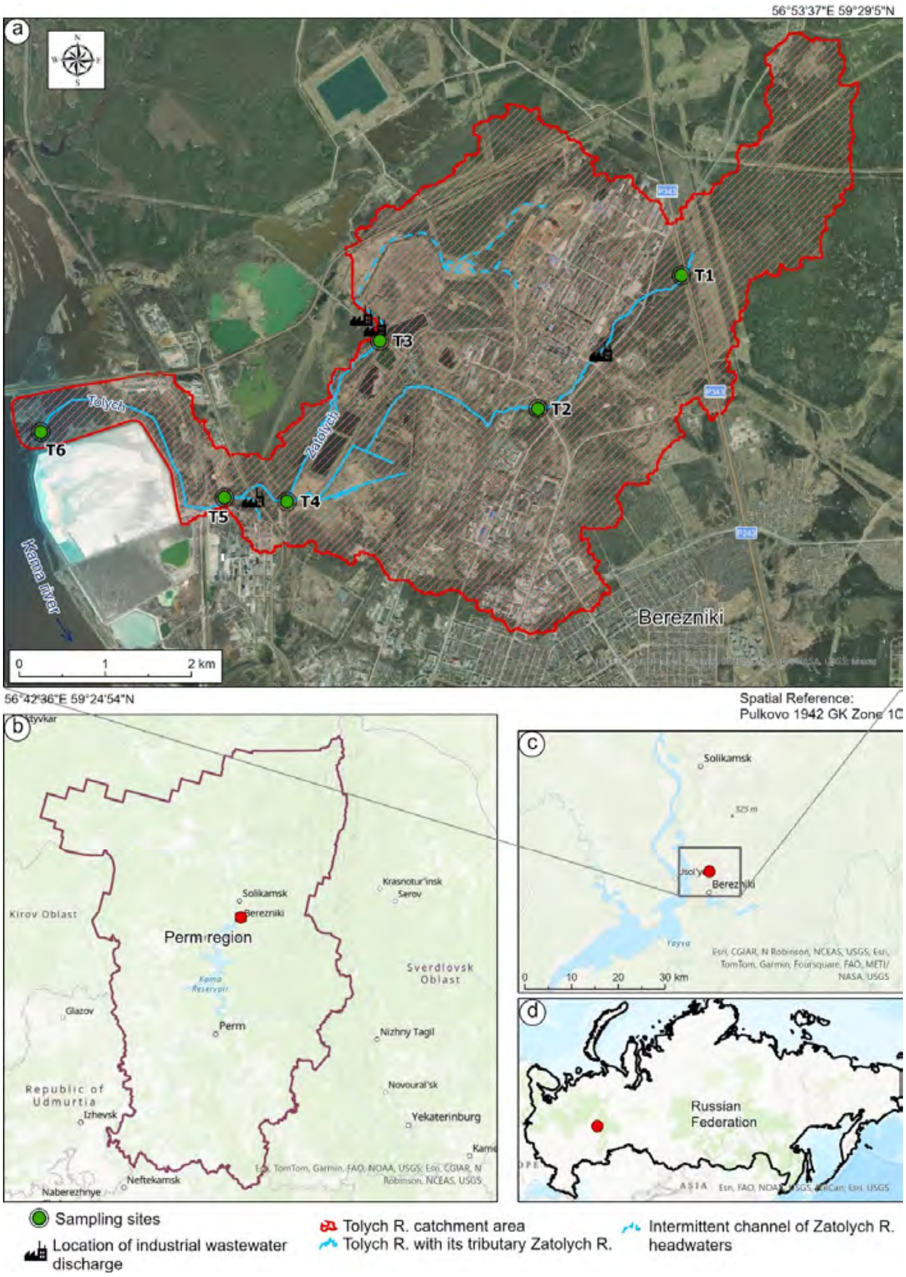


Fig. 1. Overview of the location the Tolych River and the T1 –T6 sampling sites.

Sediment samples were collected at the depth of 0.20 m. Benthic macroinvertebrates were sampled at 0.1 m^2 area by a scraper with the 20 cm wide steel blade and 500 μm mesh size. At each sampling point, invertebrates were sampled 3–4 times and then mixed into one sample. Biological material was preserved *in situ* with 4% formalin. When in the lab, invertebrates were sorted, counted, and identified by a binocular reflected light microscope (MeijiEMZ-5) to the lowest taxon-practical level by identification keys (Pankratova, 1970, 1977, 1983; Lukin, 1977; Finogenova, 1994; Starobogatov, 1995, 2000; Kluge, 1997; Zhil'tsova & Teslenko, 1997; Kanyukova, 1997; Balushkina, 1999; Makarchenko, 1999; Ivanov et al., 2001). At each sampling point, river depth, river width, and flow velocity were measured. A portable multi-parameter water quality analyzer (AP-7000, Aquaread) was used for *in situ* measurements of pH, water temperature (°C), electrical conductivity (EC), and total dissolved solids (TDS). Water samples were analyzed in the laboratory within 24 h after their collection. Their ion composition (K^+, Ca^{2+}, Mg^{2+}, Na^+, SO_4^{2-}, Cl^-, PO_4^{2-}, NO_3^-, NO_2^-) was determined by capillary electrophoresis (Capel-104, Lumex). The dissolved oxygen (DO) was analyzed by the amperometric method with a liquid analyzer Expert-001, while the titrimetric method was used to see the biochemical oxygen demand (BOD) and hydrogen carbonate ions. Aurora M90 ICP-MS spectrometer (Bruker, Fremont, CA USA) was used to assay Cr, Ni, Cu, Zn, As, Pb, Cd, and Hg concentration in bottom sediments.

Data analysis

The following biotic indices of benthic macroinvertebrates were used to evaluate water quality: the Shannon-Weaver Diversity index, the Woodiwiss biotic index, the Goodnight-Whitley oligochaete index, and the King and Ball domestic and industrial wastewater pollution index (Shannon, 1948; Goodnight & Whitley, 1961; King & Ball, 1964; Woodiwiss, 1964). The level of water pollution was assessed using threshold values from Table 1.

Threshold effect concentrations (TEC) and probable effect concentrations (PEC) were used to determine toxicity (Kownacki, Szarek-Gwiazda, 2022). Sediment samples are considered non-toxic if the measured metal concentrations are below the TEC value. If the PEC value is exceeded, the sediments are considered toxic with frequently caused adverse biological effects. Meanwhile, the toxic effects of concentrations between the PEC and TEC can be rarely observed (Macdonald et al., 2000). The toxicity of sediments in the study area was assessed using data from Table 2.

Table 1. Water pollution level by biotic indices

Category of pollution water	Indices			
	Schannon	Goodnight –Whitley	Woodiwiss	King and Ball
Non-contaminated	>3	0-59	6-10	>1
Moderate contaminated	1-3	60-79	3-5	-
Highly contaminated	<1	80-100	0-2	<1

Table 2. Sediment quality guidelines for heavy metal (Macdonald et al., 2000)

	Zn	Cd	Pb	Ni	Cu	Cr	Hg	As
TEC, mg/kg	121	0.99	35.8	22.7	31.6	43.4	0.18	9.8
PEC, mg/kg	459	4.98	128	48.6	149	111	1.06	33

The PECQ was used as a tool to assess the status of sediments based on heavy metal concentrations due to possible effects on aquatic biota (Ingersoll et al., 2001), and is calculated under the following formula:

$$PECQ = HM_{sample}/HM_{PEC}$$

$$mPECQ = \sum_{i=1}^{n} PECQ_i/nmPECQ = \sum_{i=1}^{n} PECQ_i/n \,,$$

where HM_{sample} is the measured content of the *i*-th element in sediment, HM_{PEC} is the PEC value for this element (Table 2), the mean PECQ (mPECQ) was calculated for all eight measured elements; *n* is the total number of elements. The mPECQ values are classified into three categories: (i) when mPECQ<0.1, the probability of toxicity is relatively low at 25% and; (ii) 1 < mPECQ < 5, when the sediments are considered toxic in 70-75% of cases; and (iii) mPECQ > 5, when the sediments are considered toxic with a probability of more than 75% (Ingersoll et al., 2001; Liu et al., 2017).

To estimate the correlations between the analyzed abiotic data, mPECQ values and biological data (species number, total density, biomass of invertebrates, and biotic indices), data normality was checked by the Shapiro-Wilk test. Log-transformation was used for normalization of abnormal data. To identify the relationships between the analyzed parameters, the Pearson correlation coefficient and significance of correlation relationships by 0.05 p-value were applied. Statistical analyses were performed with Past 4.12 software.

RESULTS

Hydrological and hydrochemical characteristics of the Tolych River

During the sampling period, the water velocity of the Tolych River varied from 0.05 to 0.21 m/s, stream width varied from 0.7 to 15 m, stream depth varied from 0.04 to 1.45 m (Table 3). At 2.5 km upstream of the confluence with the Kama River, the annual wastewater discharge from a chemical enterprise comprises 42,548 thousand m^3 (the average annual value from 2010 to 2019). This significantly increases the flow of the Tolych River in the lower section of the river. Thus, at the T4 sampling point located 0.65 km upstream of wastewater discharge and downstream of the confluence of the Tolych River and the Zatolych River, water discharge was

Table 3. Hydrological and hydrochemical parameters at the Tolych River catchment at the T1-T6 sampling stations

Parameter	T1	T2	T3	T4	T5	T6
Stream width, m	0.7	1.1	0.7	1.6	12.0	15.0
Stream depth, m	0.09	0.04	0.20	0.44	1.01	1.45
Water velocity, m/s	0.05	0.21	0.15	0.09	0.21	0.14
Water flow, m^3/s	0.003	0.009	0.021	0.063	2.500	3.045
Temperature, °C	24.6	24.7	19	17.6	33.3	25.1
Conductivity, µS/cm	2034	2129	676	624	440	1208
Total dissolved solids, mg/L	1936	260	712	405	356	1052
pH	6.8	6.2	5.8	5.3	7.6	7.3
PO_4^{3-}, mg/L	< 0.025	< 0.025	< 0.025	< 0.025	< 0.025	< 0.025
NH_4^+, mg/L	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5	< 0.5
NO_3^-, mg/L	0.48	0.27	3.3	3.3	1.43	0.7
NO_2^-, mg/L	< 0.02	< 0.02	< 0.02	1.25	1.14	2.4
Biological oxygen demand, mg/L	1	1.31	1	1	1.3	1.01
Dissolved oxygen, mg/L	9.6	9.2	9.2	9.2	6.9	9.2
Cl^-, mg/L	1178	99	140	172	159	622
SO_4^{2-}, mg/L	15.2	19.2	69	170	21.5	21
HCO_3^-, mg/L	54	59	293	234	71	66
Ca^{2+}, mg/L	428	35	110	123	47	207
Mg^{2+}, mg/L	36	6.2	8.7	27.2	6.9	7.4
Na^+, mg/L	140	43	102	116	62	160
K^+, mg/L	60	11.4	5.2	25.8	22.2	15.5
mPECQ	0.90	0.39	0.54	0.75	0.94	0.52

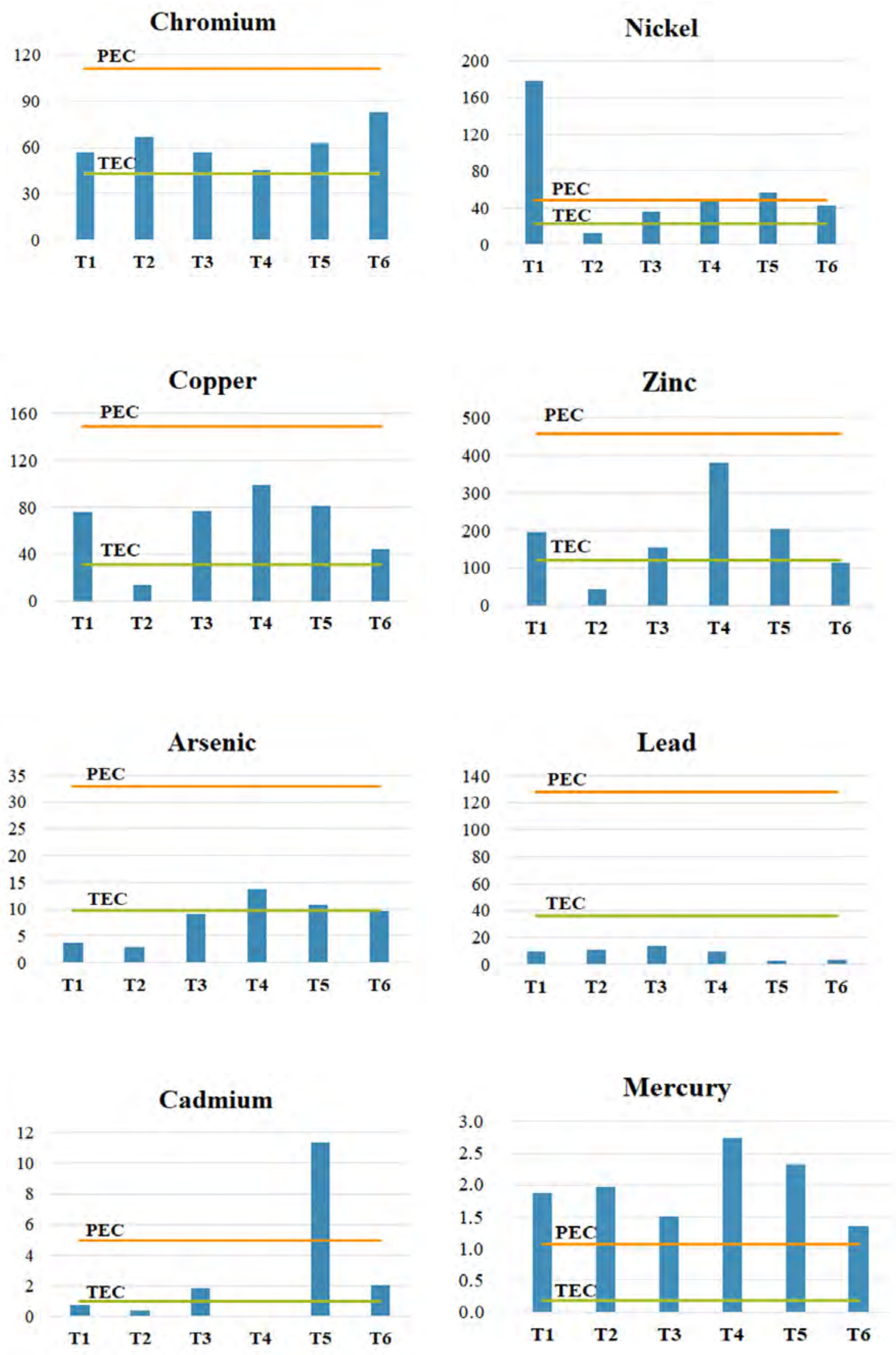


Fig. 2. Heavy metal concentrations (mg/kg, y-axis) with highlighted TEC and PEC values at the sampling points (x-axis) in the Tolych River watershed.

equal to 0.06 m^3/s, and at the T5 point (downstream of wastewater discharge), the flow increased to 2.5 m^3/s. Another source of wastewater discharge is non-ferrous metallurgy enterprises which increase the flow in the upper reach of the Tolych River by its annual value of 1,362 thousand m^3 (the average volume from 2010 to 2019). This leads to an increase in the flow from 0.003 m^3/s at the T1 sampling site located 1.3 km upstream of wastewater discharge, to 0.009 m^3/s at the T2 site located downstream of wastewater discharge. The flow of the Zatolych River is also mainly formed by the discharge of wastewater from an energy enterprise. Upstream of the T3 sampling point, the headwaters of the Zatolych River draining through the wetland area, the channel of the river is intermittent and was not distinguishable during the sampling. Significant changes in physical and chemical parameters were observed at the Tolych River downstream of the wastewater discharge from a chemical enterprise. From the T4 to T5 sampling points, the pH values changed from 5.3 to 7.6, and the temperature increased from 17.6^{o}C to 33.0^{o}C. Downstream of the T5 station, a temperature gradually decreased reaching 25.1 °C at the T6 sampling station.

Distribution of Heavy Metals in Bottom Substrate

The heavy metal concentrations in sediment samples exceeded PEC values only for Ni at the T1 and T5 sampling sites, for Cd at the T5 sampling site and for Hg at all sampling sites, which may indicate their negative impact on hydrobionts (Fig. 2). Cr, Cu, Zn concentrations were between TEC and PEC in the most of the sampling points.

The mPECQ values for bottom sediments at the T1 and T5 sites were close to 1 and can be considered toxic, while the probability of toxicity was lower at other sampling points. The lowest value of mPECQ was observed at the T2 sampling point (Table 3).

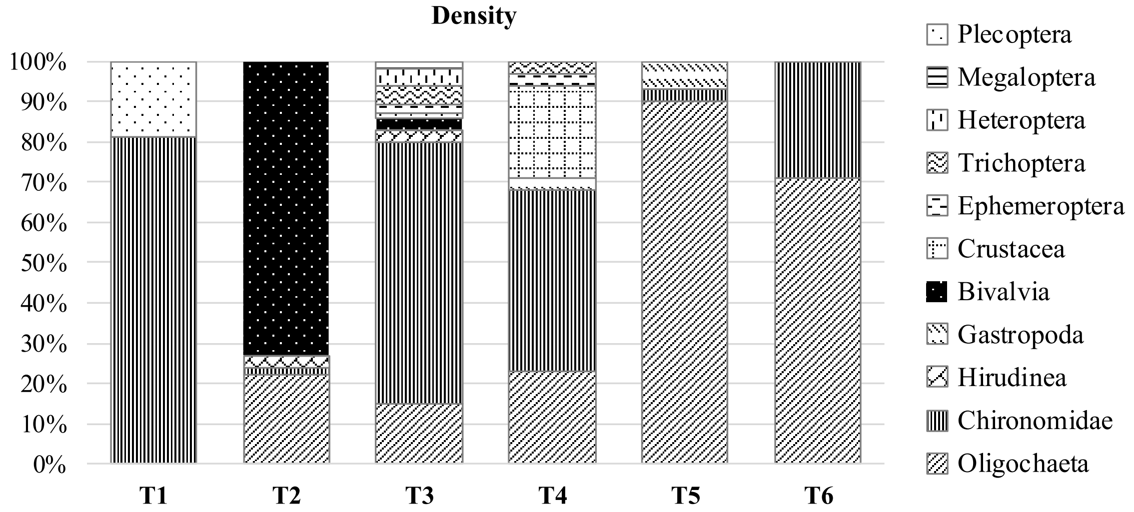
Macrozoobenthos

Macrozoobenthos of the Tolych and Zatolych Rivers was represented by 38 species and ranks higher than the species (Appendix). The density and biomass of major taxonomic groups of benthic macroinvertebrates vary among samples (Fig. 3; Table 4).

The abundance of benthic macroinvertebrates in the upper reach of the Tolych River (the T1 sampling point) was low. Chironomid larvae of *Prodiamesa olivacea* dominated at this site. Also, chironomids *Potthastia gaedi* and stoneflies *Leuctra fusca* were registered here. The total biomass and density of the macrozoobenthos was equal to 1.13 g/m^2 and 520 ind./m^2, respectively (Table 4). The values of the Woodiwiss, Goodnight–Whitley, and King and Ball biotic indices correspond to non-contaminated waters. The value of Shannon Diversity index corresponds to polluted waters.

At the T2 sampling point, the core of macrozoobentos consists of small bivalve mollusks *Conventus conventus, Hensloviana henslovana* and *Euglesa* sp. They represent up to 73% of the density and 50% of the biomass of the bottom macroinvertebrate fauna, which means 12,900 ind./m^2 and 2.76 g/m^2, respectively. The subdominants of the macrozoobenthic community are oligochaetes *Limnodrilus hoffmeisteri*. The chironomids *Chironomus plumosus* and *Thienemannimyia lentiginosa*, as well as the gastropods *Gyraulus* sp., & the leeches *Glossiphonia complanata* and *Erpobdella octoculata* are presented in minority. The values of the Shannon and Woodiwiss indices characterize the waters of this river's section as moderately polluted. The Goodnight-Whitley index corresponds to the category of clean waters, and the King and Ball index corresponds to dirty waters.

At the T3 sampling point (the Zatolych River), downstream of wastewater discharges from the energy enterprise, a relatively diverse and productive community of bottom invertebrates was formed (Fig. 2, Table 4). At this site, the core of the macrozoobenthos consists of chironomid larvae and caddisfly larvae of families Limnephilidae and Polycentropodidae, respectively. A significant proportion of animals is represented by hemipterans *Corixa affinis*, megalopterans



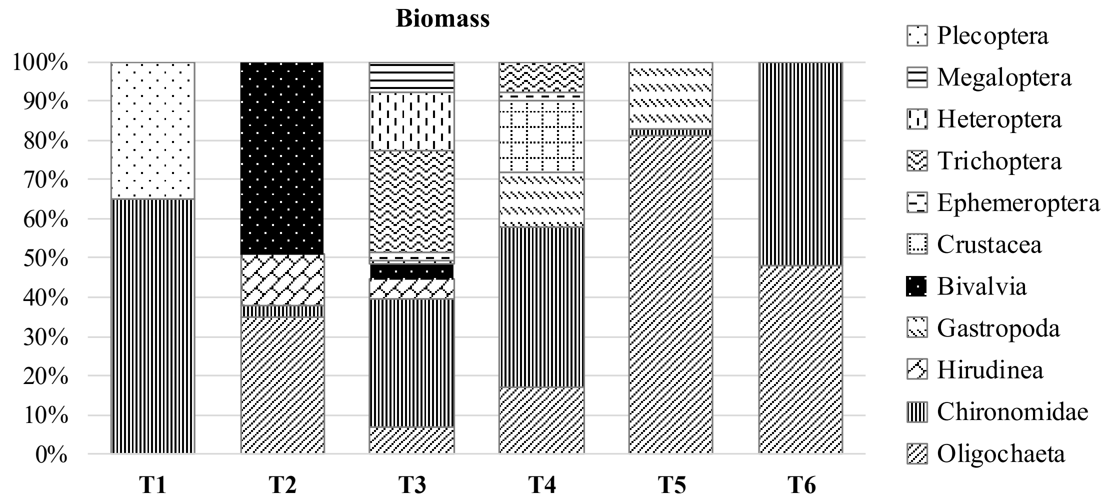


Fig. 3. Macrozoobenthos density and biomass in the Tolych River watershed by major taxonomic groups (Absolute values of density (ind./m^2) and biomass (g/m^2) are given in Table 4)

Table 4. Characteristics of macrozoobenthos at the Tolych River watershed

Parameter	T1	T2	T 3	T 4	T 5	T 6
Species number	8	10	18	14	10	9
Density, ind./м^2	520	12 900	3 250	3 220	3 120	1 460
Biomass, mg/m^2	1.13	2.76	8.35	5.1	4.37	2.39
Shannon Diversity index	2.62	2.02	3.3	3.12	1.41	1.8
Woodiwiss index	7	4	6	6	2	2
Goodnight-Whitley index	0	22	15.1	22.7	90.1	71.2
King and Ball index	∞	0.07	11.9	3.13	0.03	1.1

Sialis sordida, and leeches *Helobdella stagnalis* and *G. complanata*. They are accompanied by oligochaetes, bivalve mollusks *Euglesa* sp., larvae of mayflies *Leptophlebia submarginata*, and isopods *Asellus aquaticus*. The values of the biotic indices correspond to the category of clean waters.

Downstream of the confluence of the Tolych River and the Zatolych River (T4 sampling point), zoobenthic community is predominantly formed by chironomid larvae *Ch. plumosus* and *Microtendipes pedellus*. Also, isopods *A. aquaticus*, oligochaetes *L. hoffmeisteri*, larvae of *Baetis* mayflies, caddisflies *Hydropsyche contubernalis* and gastropods *Lymnaea truncatula* were identified. The values of biotic indices correspond to the category of clean waters.

At the T5 sampling point, 0.8 km downstream of wastewater discharges from the chemical enterprise, zoobenthocenosis was primarily (over 80% of biomass) presented by oligochaetes *L. hoffmeisteri*. They were accompanied by the gastropods *L. truncatula* and the chironomid larvae *Ch. plumosus* and *Polypedilum nubeculosum*. The values of the Woodiwiss, Goodnight-Whitley and King and Ball indices correspond to the category of dirty waters, and the small value of the Shannon index indicates a reduced diversity of benthic macroinvertebrates.

At the mouth of the Tolych River (T6), core elements of zoobentocenosis were represented by chironomids *Ch. plumosus*, *P. nubeculosum* and *Procladius ferrugineus*, as well as oligochaetes *L. hoffmeisteri*. The biomass and density of benthic animals here were reduced in comparison to upstream located sites (Table 4). The low value of the Shannon information index indicates a simplified structure of the community, and the King and Ball, Woodiwiss and Goodnight-Whitley biotic indices correspond to the category of dirty waters.

With several exceptions, correlation analysis did not show any significant associations between biotic data and abiotic variables (Table 3, 4). Several cases of significant positive correlations and one case of negative correlations were revealed. The significant negative association between abiotic and biotic variables were observed between temperature and the Shannon Diversity index ($r_{(6)} = -.95, p = .003$). Significant positive correlations were observed between the concentration of NO_3^- and HCO_3^- ions and the number of benthic invertebrate species (NO_3^-, $r_{(6)} = .90, p = .01$; HCO_3^-, $r_{(6)} = .97, p = .001$), its total biomass (NO_3^-, $r_{(6)} = .87, p = .01$; HCO_3^-, $r_{(6)} = .90, p = .001$) and the Shannon Diversity index values (HCO_3^-, $r_{(6)} = .83, p = .04$). Apart from that, significant positive correlations were revealed between the values of the King and Ball index and concentration of major inorganic ions (Cl^-, $r_{(6)} = .88, p = .02$; Ca_2^+, $r_{(6)} = .91, p = .01$; K^+, $r_{(6)} = .89, p = .02$) and TDS values ($r_{(6)} = .90, p = .01$). Heavy metal concentrations, mPECQ values, and biotic data showed no significant correlations.

DISCUSSION

The results show that the Tolych River is mainly polluted by the chemical industry enterprise located 2.5 km upstream of the river mouth, which leads to changes in bottom macroinvertebrate fauna. The wastewater discharge averaged 42.5 million m^3 from 2010 to 2019 or approximately 1-1.5 m^3/sec, which is greater than the annual river flow. The inflow of wastewaters decreases the concentration of major inorganic and organic ions. At the same time, there is a significant increase in temperature and a decrease in the concentration of dissolved oxygen in water, which contributes into the degradation of benthic communities. Downstream of the chemical plant's wastewater discharge, macrozoobenthos is characterized by a simplified structure. It predominantly consists of pollution-tolerant oligochaetes of the family Tubificidae, which account for 71-90% of the total abundance of bottom fauna, while the groups of animals sensitive to water quality are completely absent. The biotic index values of this river section correspond to the dirty waters. This demonstrates a well-known negative impact of higher temperatures and oxygen depletion on benthos of boreal streams and correlates with the observed significant negative associations between temperature and the Shannon Diversity index (Živić et al., 2006; Quevedo et al., 2018).

Cadmium concentration at the T5 site can be another factor which negatively affects macrozoobenthos. The input of a significant volume of wastewater in the lower part of the Tolych River catchment contributes to a decrease in the concentrations of most studied heavy metals from the T4 to T6 sites, except for Cd, which showed a 640-time increase at the T5 site compared to the T4 sampling point. High Cd could be explained by the increase in chloride coming from the effluents of the chemical plant in the average volume of 3,448 tons per year, which can cause the release of cadmium ions from humate complexes in bottom sediments (Ondrasek et al., 2022). Cadmium in bottom sediments at the T5 point amounted to 11.3 mg/kg. It is known that cadmium is one of the most toxic and hazardous trace elements, and its higher concentration in sediments combined with thermal pollution could be a significant factor leading to the impoverishment of benthic fauna and loss of EPT invertebrates which are sensitive to pollution (Brix et al., 2017). However, the Cd pollution level was stabilized towards the mouth of the river, where Cd concentration was below PEC values.

The excess of mercury concentration in sediments relative to the PEC level throughout the Tolych River, as well as local exceedances in the number of heavy metals above the PEC and TEC levels, are able to lead to chronic negative impact on macroinvertebrates (Long et al., 2006; Kolarova & Napiórkowski, 2021). Apart from the toxic effect of Cd mentioned above, joint exposure to high concentrations of cadmium and mercury can lead to a synergistic increase in the toxic effects of these elements on macroinvertebrates, that was demonstrated with the bivalve *Perna viridis* (Bezmaternykh, 2018). The significant PEC exceedance was noted at the T1 sampling point for nickel with 178.5 mg/kg in sediments. At the same time, a stoneflies larvae *L. fusca* was found at this location. The presence of stoneflies sensitive to water quality can be explained by both the low toxicity of nickel compared to other heavy metals and by the differentiated sensitivity of various invertebrates to specific pollutants. The TEC and PEC values widely used in toxicological works were calculated by biotesting of North American amphipod *Hyalella azteca* and chironomids *Chironomus tentans* and *C. riparius* (Mohan et al., 1986). However, more recent experiments with amphipods (*H. azteca, Gammarus pseudolimnaeus*), mayflies (*Hexagenia sp.*), oligochaetes (*Tubifex tubifex, Lumbriculus variegatus*), mollusks (*Lampsilis siliquoidea*), and chironomid larvae (*C. dilutus, C. riparius*) demonstrated that the threshold concentrations of nickel exposure in sediments can vary from 126 to 281 mg/kg depending on the species, as well as changes in the toxic effect when nickel is combined with other pollutants (Ingersoll & MacDonald, 1999). Tolerance of different groups of macroinvertebrates to mercury exposure is even more variable. Analysis of macroinvertebrates in sediment samples with mercury content from 0.27 to 183 mg/kg revealed that *Placobdella* leeches significantly decreased in abundance and biomass with higher mercury concentration, while oligochaetes and chironomids *Procladius* demonstrated the opposite pattern. At the same time, the response of invertebrates to mercury in sediments also depended on environmental factors, in particular the fractional composition of the sediment. For example, the abundance of chironomids *Chironomus* decreased with both higher sediment fraction and higher mercury concentration (Vangheluwe et al., 2013). The influence of sediment fraction was also found for the amphipod *Rhepoxynius abronius*, whose mortality decreased to 1% when a fine fraction was added to a bottom substrate containing 13 mg/kg mercury (Suchanek et al., 1995). In this regard, toxicity assessment of complex pollution should cautiously refer to TEC and PEC values due to changes in toxic effects driven by a combination of different pollutants, environmental characteristics, and local tolerance of invertebrates.

The complex pollution of the Tolych River is characterized by various chemical variables which can bring both synergistic and antagonistic effects of toxicity of specific pollutants. For instance, low pH values observed in the river can lead to desorption of heavy metals from sediments, their higher solubility in water and availability to benthic fauna (Khayrulina, 2022). This often promotes the toxic effect of heavy metals on invertebrates, which, in particular, was revealed in the effect of copper ions on bivalves (Cao et al., 2019). In addition, sampling revealed organic pollution of the Tolych River. There is an excess of nitrite ions by tens of times compared to the level of maximum concentrations permissible for fisheries water bodies. Nitrite is toxic to gill-breathing invertebrates, particularly crustaceans (Alonso & Camargo, 2006). The main toxic effect is that the oxygen-transmitting pigment hemocyanin in the hemolymph of crustaceans transforms into a form of methemocyanin, which cannot bind with oxygen, resulting in asphyxiation and death of the animal (Cheng & Chen, 2000; Jensen, 2003). However, with higher chloride concentration, the toxic effect of nitrite ions is decreased. After diffusing through the gill epithelium, chloride ions and nitrite ions use the same pathway, acting as competitive inhibitors (Alonso & Camargo, 2008). This may explain the presence of *A. aquaticus* at the T4 sampling point, where increased nitrite-ion content was observed in a chloride-ion concentration of 172 mg/L. At the same time, it was experimentally proved that an increase in chloride ion concentration from 27.8 to 108 mg/l significantly reduced the toxic effect of exposure to nitrite ion with a concentration of 5 mg/l for the amphipod *Eulimnogammarus toletanus* (Camargo & Alonso, 2006).

The results of correlation used for the analysis of associations between biotic data and abiotic variables demand additional discussion. Significant positive correlations are found between concentration of major ions, TDS values, and the number of benthic macroinvertebrate species, its total biomass, the Shannon Diversity and King and Ball indices. The concentrations of analyzed ions, except for Cl^- ion, were below permissible levels, and potentially revealed correlations reflect habitat differentiation of a sampling site rather than the direct effect of these ions on benthos. The same explanation can be used for interpreting the results of positive correlations between the King and Ball index and the concentration of Cl^- ion and TDS values. The highest values of these parameters were 1,178 mg/l and 1,352 mg/l respectively, and according to our unpublished data, the level of mineralization leading to degradation of water quality in the streams of Perm Krai ranges between 5,500 and 7,500 mg/l by the King and Ball index, which is far above values registered in the Tolych River during the sampling.

Habitat heterogeneity could be taken as a major factor for the changes in water quality assessment based on different biotic indexes. At the T1 site, the presence of stoneflies demanding high water quality (Aleksander-Kwaterczak & Plenzler, 2019), as well as larvae of the Orthocladiinae subfamily which is also sensitive to pollution (Newman et al, 2021), indicate the good quality of the water. This is confirmed by the values of the Woodiwiss, Goodnight-Whitley and King and Ball biotic indices, which correspond to the category of clean waters. At the same time, the Shannon index showed that water quality is moderately polluted. However, the simplification of the community structure can be caused not only by pollution, but also by the uniformity of bottom substrate (Andrianova, 2015). Indeed, the relatively homogeneous sandy bottom substrate of this biotope, the poverty of algae fouling, and the insignificant accumulation of detritus do not allow to expect the formation of a rich and diverse macrozoobenthic community here. The same reason can be used to explain the results from the T2 site where the values of the Shannon and Woodiwiss indices characterize the waters of this river section as moderately polluted, but the Goodnight-Whitley index corresponds to the category of clean waters, and the King and Ball index corresponds to dirty waters. Discrepancies in water quality estimates under different biotic indices may be also related to the specificity of the bottom substrate here. Sandy soil with moderate siltation is a poorly structured environment that causes the development of a community with reduced biological diversity. The absence of rigid fractions in this type of substrate excludes the development of lithophilic animals with the least tolerance to pollution. Both factors lead to low values of the Shannon and Woodiwiss indices. The low value of the King and Ball index at the T2 site is determined by the small proportion of insects in the total biomass of macrozoobenthos. This can be explained not only by pollution, but also by biotic relations within the community, in particular, intense competition of insects with other benthic macroinvertebrates, especially predatory invertebrates – leeches, whose proportion in the biomass of the bottom fauna is 13%. Perhaps, this factor is associated with the abundance of small bivalve mollusks in this section of the river, which are inaccessible to leeches and, consequently, gaining a competitive advantage here. In addition to the value of the Goodnight-Whitley index, the rich development of *C. conventus* mollusks typical for oligotrophic conditions (Kornyushin, 1996) and the lowest value of mPECQ also correspond to the good water quality in this section of the river.

CONCLUSION

Differences in species composition and ratio of benthic macroinvertebrates subdivide the Tolych River into two sections upstream and downstream of the T5 sampling point, the boundary between which is marked by the chemical plant's effluent discharge point. Upstream of the T5 sampling station, there are benthic communities with various groups of invertebrates, including water quality demanding bivalves and insects, and the proportion of oligochaetes in the invertebrates' abundance did not exceed 22.7%. The values of biotic indices calculated for these benthic macroinvertebrates show that the waters of this river section can be categorized as clean. Downstream of the chemical plant's wastewater discharge, zoobenthic communities characterized by a simplified structure. The macrozoobenthos predominantly consisted of pollution-tolerant oligochaetes of the family Tubificidae, which account for 71-90% of the total abundance, while groups of animals demanding water quality were completely absent. The biotic index values of this river section correspond to the dirty waters.

The probability of negative impact of heavy metals in sediments on invertebrates was derived from the used abiotic indices. Higher mercury concentration can be considered as the most serious ecological risk for macroinvertebrates throughout the river. The biological impact probability value (mPECQ) indicates a high degree of toxic risk to benthic fauna in the upper reaches of the river. These results contradict the ones obtained from the biotic indices. Apart from that, our results show that in the middle section of the river, toxic effect of a high nitrite-ion concentration contradicts the presence of crustaceans sensitive to this type of pollution. This can be explained by the antagonistic effect of the high chloride concentration at this site.

Finally, abiotic indices used in this study and widely used in toxicological work can show results different from the ones based on biotic data. As a recommendation, toxic effects of pollutants on macroinvertebrates should be assessed with regard to the complexity of pollution, the dynamics of environmental factors, and local tolerance of invertebrates. An important part of future studies on aquatic ecosystem health should be focused on the development of regional biotic and abiotic indexes. The conducting of aquatic ecosystems pollution analysis should be based on a multidisciplinary approach which effectiveness arising from the recent studies (Markowski & Wojtasik, 2024).

DECLARATION OF COMPETING INTEREST

The authors declare that there is not any conflict of interests regarding the publication of this manuscript. In addition, the ethical issues, including plagiarism, informed consent, misconduct, data fabrication and/ or falsification, double publication and/or submission, and redundancy has been completely observed by the authors.

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AUTHOR CONTRIBUTIONS

Pavel B. Mikheev: Writing – Reviewing and Editing, Supervision. Nikolay N. Pankov:

Data curation, Methodology, Writing – Original draft preparation. Evgeniya S. Ushakova: Data curation, Methodology, Writing. Mikhail A. Baklanov: Data curation, Methodology. Elena V. Drobinina: Visualization, Methodology, Software. Alexey Yu. Puzik: Data curation, Methodology. Margarita A. Volkova: Data curation, Methodology.

LIFE SCIENCE REPORTING

No life science threat was practiced in this research.

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APPENDIX

The list of macrozoobenthos species and ranks higher than the species registered in the sampled locations of the Tolych River and Zatolych River

Taxon	T1	T2	T3	T4	T5	T6
Oligochaeta						
Nais pseudoptusa Piguet, 1906			+			
Nais variabilis Piguet, 1906			+			
Ophidonais serpentina (O.F.Mueller, 1773)			+			
Limnodrilus hoffmeisteri Claparede, 1862		+	+	+	+	+
Limnodrilus udekemianus Claparede, 1862				+	+	+
Limnodrilus claparedeanus Ratzel, 1868				+	+	+
Tubifex tubifex (O.F.Mueller, 1773)				+	+	+
Hirudinea						
Erpobdella octoculata (Linnaeus, 1758)		+				
Glossiphonia complanata (Linne, 1758)		+	+		+	
Helobdella stagnalis (Linne, 1758)			+		+	
Gastropoda						
Gyraulus sp.		+				
Lymnaea truncatula (Mueller, 1774)				+	+	
Bivalvia						
Euglesa sp.		+	+			
Conventus conventus (Clessin, 1877)		+				
Hensloviana henslovana (Sheppard, 1823)		+				
Crustacea						
Asellus aquaticus (Linne, 1758)			+	+		
Ephemeroptera						
Baetis fuscatus (Linnaeus, 1761)				+		
Baetis scambus Eaton, 1870				+		
Baetis vernus Curtis, 1830	+			+		
Leptophlebia submarginata (Stephens, 1835)			+			
Plecoptera						
Leuctra fusca (Linnaeus, 1758)	+					
Nemoura cinerea (Retzius, 1783)	+					
Protonemura intricata (Ris, 1902)	+					
Heteroptera						
Corixa affinis Leach, 1817			+			
Trichoptera						
Hydropsyche contubernalis McLachlan, 1865				+		
Limnephilus rhombicus (Linnaeus, 1758)			+			
Phryganea grandis Linnaeus, 1758		+				
Polycentropus flavomaculatus Pictet, 1834			+			
Megaloptera						
Sialis sordida Klingstedt, 1932			+			
Diptera: Chironomidae						
Chironomus plumosus (Linne, 1758)		+	+	+	+	+
Cricotopus sp.	+					
Cryptochironomus defectus Kieffer, 1921			+			
Microtendipes pedellus (De Geer, 1776)		+	+	+	+	+
Polypedilum nubeculosum (Meigen, 1818)		+	+	+	+	+
Potthastia gaedi (Meigen, 1838)	+					
Procladius ferrugineus Kieffer, 1919		+	+	+		+
Prodiamesa olivacea (Meigen, 1818)	+					
Thienemannimyia lentiginosa (Fries, 1823)	+					+