





27 **Abstract**

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29 Crustaceans and annelids are key components of groundwater communities, influenced
30 by both abiotic conditions and biotic interactions. This study assessed their diversity in urban
31 groundwaters accessed via 91 dug/drilled wells in Kraków, southern Poland, subject to
32 chronic anthropogenic disturbance. Invertebrates were recorded in 47 wells, with 19 species-
33 group taxa identified from 28 wells, including eight annelid and 11 crustacean taxa
34 (Ostracoda: 3; Copepoda: 6; Bathynellacea: 1; Amphipoda: 1). Six **stygobiontic** taxa were
35 detected in 10 wells: *Trichodrilus cernosvitovi*, *Trichodrilus* sp., *Typhlocypris* cf. *eremita*,
36 *Diacyclops languidoides*, *Bathynella natans*, and *Niphargus* cf. *tatrensis*. Due to some
37 taxonomic uncertainties, open nomenclature was used where necessary. Species accumulation
38 did not reach saturation, but extrapolation suggested the sampling was **near-complete**. Alpha
39 diversity was low (1–3 species per well, mean = 1.4), while beta diversity was high
40 (Whittaker index = 12.3), indicating substantial species turnover, a typical feature of
41 groundwater ecosystems. No clear seasonal trends were observed, consistent with previous
42 studies in Kraków. Four main community types were identified. One, dominated by
43 *Enchytraeus* gr. *buchholzi*, may indicate degraded conditions, another, with *Bathynella natans*
44 and *Aeolosoma* spp., suggests transitional states; a third, dominated by *Trichodrilus* spp.,
45 likely reflects relatively undisturbed groundwater; and a fourth, more heterogeneous type
46 dominated by surface copepods, was ecologically ambiguous. Despite generally low richness
47 and dominance by surface taxa, the presence of six stygobiontic species suggests that at least
48 20% of the surveyed wells retain relatively good ecological conditions.

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51 **Keywords:** subterranean waters, municipal wells, stygobionts, expected species richness

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55 1. Introduction

56

57 Urban wells, constructed for various purposes, provide convenient access to
58 groundwater and offer valuable opportunities for studying subterranean aquatic fauna. Urban
59 expansion is a global phenomenon, with cities continuously increasing in area (Kraków in
60 southern Poland, for instance, has expanded by 42% since 1950), often encompassing regions
61 of high conservation value. In urban settings, groundwater is frequently exposed to various
62 types of pollution, including chemical, organic, and thermal contaminants (Kim, 1992; Burri
63 et al., 2019; Becher et al., 2022), which pose significant risks to subterranean fauna. These
64 threats are increasingly recognized, prompting frequent assessments of water chemistry and
65 microbial communities. Consequently, the monitoring of groundwater quality has become a
66 standard practice in many European countries under the EU Water Framework Directive
67 (WFD, 2000) and the Groundwater Directive (GWD, 2006).

68 Despite the ecological significance of urban groundwater ecosystems, the invertebrate
69 fauna of urban wells has historically been studied in only a limited number of European cities.
70 Some of the earliest and most extensive research was conducted in Prague, Czech Republic,
71 by Vejdovský (1882), Sládeček and Řehačková (1952), Řehačková (1953), and Ertl (1957).
72 Additional historical studies include those by Jaworowski (1893) in wells of Lviv (Ukraine)
73 and Kraków, Chappuis (1924) in Basel, and Vornatscher (1972) on crustaceans in Vienna.
74 After a long hiatus, interest in urban groundwater fauna has recently resurged, with studies by
75 Koch et al. (2021), Becher et al. (2022, 2024), Englisch et al. (2024), and Meyer et al. (2024).
76 Only very recently have systematic efforts to monitor groundwater fauna in cities been
77 initiated (Johns, 2024), underscoring the growing importance of this field. Earlier publications
78 focused primarily on species inventories, whereas recent studies have increasingly addressed
79 the impacts of urbanization and patterns of biodiversity.

80 The most commonly encountered invertebrate groups in urban wells are annelids and
81 crustaceans. In Poland, annelids have been relatively well studied in various subterranean
82 habitats, including rural wells, and additional records have been provided in the course of
83 other ecological research. These data were comprehensively reviewed by Dumnicka et al.
84 (2020). A checklist of Polish groundwater crustaceans was compiled by Pociecha et al.
85 (2021), and subsequently complemented by Karpowicz et al. (2021) and Karpowicz and
86 Smolska (2024). The distribution of the amphipod genus *Niphargus* in Poland has been
87 particularly well documented, primarily through the work of Skalski (e.g., 1970, 1981), with a



88 synthesis provided by Dumnicka and Galas (2017) and additional records recently reported
89 (pers. comm. A. Górny).

90 Despite this progress, the subterranean aquatic fauna of urban areas in Poland remains
91 insufficiently explored. In Kraków, local geology and contamination patterns have been
92 shown to influence species presence and distribution (Dumnicka et al., 2025).

93 The aim of this study was to assess the taxonomic diversity of annelid and crustacean
94 communities inhabiting groundwater from 91 urban wells in Kraków, estimate total species
95 richness, evaluate seasonal variation, and identify major community types. These insights
96 may help determine whether groundwater in at least some of these wells still retains a
97 reasonably good ecological state.

98

99 **2. Material and Methods**

100

101 **2.1. Study area and sampling sites**

102 Kraków is located at the junction of three tectonic units: the Silesian-Kraków
103 Monocline, the Miechów Basin, and the Precarpathian Depression, which results in a highly
104 complex geological and hydrogeological structure of this area (Kleczkowski et al., 2009). The
105 studied urban wells are distributed across the city, from the historic Old Market Square to the
106 outer residential districts (Fig. 1). Most wells are situated in Quaternary sediments composed
107 of gravel and sand, with occasional inclusions of peat and silt. In the city center, these are
108 overlain by a few-meter-thick layer of anthropogenic deposits. Other wells are located in
109 Neogene (Miocene) sediments composed of various lithologies, such as marls, shales, and
110 gypsum (gypsum-salt formations) (Rutkowski, 1989). The Vistula River flows through
111 Kraków center and is fed by several tributaries (Fig. 1). Holocene fluvial sediments, mainly
112 sands and gravels, occur within the river valleys.

113 According to the list obtained from the Kraków Water Company, approximately 350
114 bored/dug, driven or drilled wells were constructed in the area between the early 19th century
115 and the 1980s. Currently, only about half of these are operational. For the present study, 83
116 such relatively shallow bored/dug wells were selected. Their depths range from 2.3 to 30.0
117 meters, with a typical water column height of 2–4 meters. These wells are primarily fed by
118 percolating water from Quaternary aquifers; however, hydraulic connections with deeper
119 Jurassic or Cretaceous layers have been identified in some locations (Chowaniec et al., 2007).
120 Each well is equipped with a piston pump and is fully sealed at the surface (Fig. 2, left).



121 In addition, 11 deep artesian wells are present in the area. These wells reach the
122 Jurassic aquifer at depths of 80–100 meters and discharge groundwater to the surface without
123 mechanical pumping due to artesian pressure (Rajchel, 1998). The emerging water is directed
124 through pipes (Fig. 2, right). Five of these artesian wells were included in the study.

125 Finally, tap water samples were collected from three separate municipal water intakes.
126 Although well water is not potable, it is occasionally used for purposes such as plant
127 irrigation.

128

129 2.2. Sampling, sample processing and invertebrate identification

130 Sampling was conducted in two consecutive years, 2019 (59 wells) and 2020 (32 wells),
131 with two sampling events per year: spring (April–June) and autumn (September–October). At
132 each of the 91 sampling sites (see Supplementary Table 1 and Fig. 1) and during each
133 sampling event, 100 L of groundwater were filtered using a plankton net with a mesh size of
134 50 µm. Invertebrates were sorted live under a stereoscopic microscope and subsequently
135 preserved in 95% ethanol. Crustaceans and annelids were identified to the species level
136 whenever possible, based on available literature (e.g., Meisch, 2000; Timm, 2009; Błędzki
137 and Rybak, 2016). All taxa were analyzed in samples from both sampling years, with the
138 exception of copepods, which were identified to species level only in the samples collected
139 during the second year.

140 Water **properties** were measured concurrently with biological sampling. Temperature
141 and specific conductivity (at 25°C) were recorded *in situ* using a portable multimeter
142 (Elmetron CX-401). Ion concentrations were determined in the laboratory of the Institute of
143 Geography and Spatial Management, Jagiellonian University, Kraków, Poland, using ion
144 chromatography following the methods described in Dumnicka et al. (2025).

145

146 2.3. Ecological and statistical analyses

147 To assess whether the sampling effort was sufficient to capture the **theoretical** species
148 richness of the studied area, species **accumulation** curves were generated using all 182 samples
149 collected from the 91 wells. These curves illustrate how the number of detected species
150 increases with the number of accumulated samples. Four standard non-parametric richness
151 estimators based on abundance data (Chao 1, Jackknife 1, Bootstrap, and Michaelis-Menten)
152 were also calculated to predict the total expected species richness. The mean (and standard
153 deviation for Chao 1) of both observed and estimated species richness were calculated from
154 9,999 permutations, with samples added in random order, using PAST v. 4.10 (Hammer et al.,



2001) and the Species-Accumulation Plot routine in PRIMER 7 software (Clarke and Gorley, 2015).

To evaluate biodiversity across the wells, several diversity metrics were computed using PAST v. 4.10 (Hammer et al., 2001) and in PRIMER 7 (Clarke and Gorley, 2015). These included: a) Alpha diversity (α) – species richness per site, b) species frequency – number of wells in which each species was present, c) **Gamma** diversity (γ) – total species richness across all sites, and d) **Beta** diversity (β) – computed as Whittaker's species turnover index ($\beta = \gamma / \alpha - 1$). For both alpha diversity and species frequency, mean values, ranges, standard deviations, and 95% confidence intervals were estimated using the bias-corrected and accelerated (BCa) bootstrap method with 9,999 replicates. These diversity metrics were calculated separately for three datasets: 1) all species, 2) **stygobiontic** species, and 3) non-**stygobiontic** species. Differences in mean alpha diversity and mean species frequency between **stygobiontic** and non-**stygobiontic** groups were tested using one-way permutational analysis of variance (PERMANOVA) with 9,999 permutations in PRIMER 7 with the PERMANOVA+ add-on (Anderson et al., 2008).

To assess seasonal differences in the composition and structure of crustacean and annelid communities, non-metric multidimensional scaling (nMDS) was performed based on Bray-Curtis similarity matrices derived from species abundance data (with all-zero samples excluded). Seasonal differences were further tested using PERMANOVA (9,999 permutations), implemented in PRIMER 7 with the PERMANOVA+ add-on.

Finally, Principal Coordinates Analysis (PCoA) based on Bray-Curtis similarity of relative abundance (percentage) data was used to visualize differences in community structure and composition of crustaceans and annelids among wells. Pearson correlation vectors (threshold > 0.2) were overlaid on the PCoA plot to highlight taxa contributing most strongly to observed patterns. This analysis was also conducted using PRIMER 7 with the PERMANOVA+ add-on.

181

182 **3. Results**

183

184 **3.1. Groundwater physical and chemical properties**

A summary of the variation in physical and chemical water properties across the studied wells is provided in Fig. 3, with detailed data available in Dumnicka et al. (2025).

Water temperature in all wells was relatively high, ranging from 9.7 to 16.2°C, while pH values were predominantly circumneutral (6.5–8.2). Electrical conductivity ranged from 314 to



189 3641 $\mu\text{S}/\text{cm}$, with a mean of 1276 $\mu\text{S}/\text{cm}$, indicating soft to moderately hard water and generally
190 low to moderate dissolved ion concentrations. Some wells exhibited elevated concentrations of
191 sulphates and/or chlorides ($> 250 \text{ mg/L}$), with values varying depending on well location and
192 local pollution sources (Dumnicka et al., 2025). Nitrogen concentrations, in the form of nitrates
193 and ammonium, also varied widely. A considerable proportion of wells were nitrate-enriched
194 ($> 50 \text{ mg NO}_3^-/\text{L}$) or ammonium-enriched ($> 0.5 \text{ mg NH}_4^+/\text{L}$). Additionally, relatively high
195 concentration of phosphates ($> 0.1 \text{ mg PO}_4^{3-}/\text{L}$) and of fluorides ($> 1.5 \text{ mg F}^-/\text{L}$) were recorded
196 in some wells (see Dumnicka et al., 2025; Fig. 3 here).

197

198 3.2. Taxonomic richness and diversity

199 Crustaceans or annelids were recorded in 47 out of 91 wells (52%), but these could be
200 identified to species level in 28 wells (31%), resulting in 19 species-group taxa (gamma
201 diversity) belonging in Annelida and in four crustacean groups: Ostracoda, Copepoda,
202 Bathynellacea, and Amphipoda, and representing both stygobiontic and non-stygobiontic
203 ecological groups. Interestingly, annelids and crustaceans occurred largely allopatrically
204 (Table 1). Other invertebrate taxa, including Microturbellaria, Nematoda, Rotifera,
205 Collembola, and Diptera larvae, were also found but were excluded from further analysis as
206 they were not identified to species level (see Dumnicka et al., 2025).

207 As expected given the rarity of many species, the species accumulation curve of the
208 observed number of annelid and crustacean species based on all 182 samples from 91 wells
209 did not reach an asymptote (Fig. 4), indicating incomplete sampling. Extrapolated species
210 richness estimates ranged from 23.2 (Bootstrap) to 31.5 (Chao 1), suggesting that between
211 60.3% and 81.9% of the estimated total species pool of the studied area was captured (Table
212 2).

213 Annelids were found in 17 wells (Table 1), and conservatively assigned to at least
214 eight species. Many specimens were immature and were therefore left in open nomenclature.
215 The semi-aquatic family Enchytraeidae was considered as represented by two species: one
216 identified as *Enchytraeus* gr. *buchholzi* and one taxon of the genus *Achaeta*. Other recorded
217 annelids included surface-dwelling *Rhynchelmis* (Lumbriculidae), treated as a single species
218 due to immature material, two *Aeolosoma* species-group taxa, one identified as *A. hyalinum*,
219 and stygobiontic *Trichodrilus*, conservatively treated as two species-group taxa, one of which
220 (*T. cernovitovi*) was confirmed by a single mature individual.

221 Ostracods were recorded only in three wells and they were represented by at least three
222 species of the family Candonidae, one stygobiontic *Typhlocypris* cf. *eremita*, and two other



223 surface dwelling species left in open nomenclature since the collected juvenile specimens
224 were not identified down to the species level (representatives of the genus *Pseudocandona*)
225 and neither at the species nor at the genus level (representatives of the family Candonidae)
226 (Table 1).

227 Copepods were the most frequently recorded group, present in 37 wells. However,
228 only specimens from the second sampling year were identified to species level and considered
229 in further analyses. Six species were identified, most typical of small, astatic surface waters.
230 Only one **stygobiontic** species, *Diacyclops languidoides*, was recorded in a single well (Table
231 1).

232 Two additional **stygobiontic** crustaceans were recorded: *Bathynella natans*
233 (Bathynellacea) and *Niphargus* cf. *tatrensis* (Amphipoda) (Table 1).

234 Among the 28 wells where crustaceans and/or annelids were identified to species level,
235 the most frequent species were *Enchytraeus* gr. *buchholzi* (found in 10 wells, 36%) and
236 *Trichodrilus* spp. (4 wells, 14%). Notably, 10 species (53%) were found in only one well
237 (Table 1). The mean species occurrence frequency across the full dataset was 2.1 ± 2.0 wells
238 (mean \pm standard deviation SD), with no significant difference between stygobionts ($2.0 \pm$
239 1.3) and non-stygobionts (2.2 ± 2.5) (PERMANOVA: $F = 0.020$, $P = 0.953$; Table 3).

240 Alpha diversity averaged 1.4 ± 0.7 species per well, ranging from 1 to 3. **Stygobiontic**
241 species exhibited significantly lower mean alpha diversity (0.4 ± 0.7) compared to non-
242 stygobionts (1.0 ± 0.7) (PERMANOVA: $F = 4.874$, $P = 0.026$). Despite generally low alpha
243 diversity, beta diversity (Whittaker index) was high for the entire dataset (12.3) and for both
244 stygobionts (13.0) and non-stygobionts (12.0), indicating high species turnover among wells
245 (Table 3). This is consistent with the PCoA results based on Bray-Curtis similarity for each
246 pair of wells (see below).

247

248 3.3. Seasonal variation in community structure and composition

249 Non-metric Multidimensional Scaling (nMDS) ordination revealed overlap between
250 samples collected in spring and autumn (Fig. 5), suggesting no significant seasonal
251 differences in the structure and composition of crustacean and annelid communities. This was
252 supported by PERMANOVA ($F = 1.30$, $P = 0.171$).

253

254 3.4. Major community types

255 Principal Coordinate Analysis (PCoA) revealed four distinct community types, with
256 the first two axes explaining 35.7% of the total variance (Fig. 6). Eight species/taxa exhibited



the strongest correlations with the first two PCoA axes and contributed most to the observed pattern (Table 4). The first PCoA axis separated seven wells dominated by nearly monospecific community of *Enchytraeus* gr. *buchholzi* (with minor contribution from Candonidae ostracods), positioned on the right side of the plot, from three additional community types on the left. The lower-left quadrant grouped four wells dominated by stygobiontic *Trichodrilus* spp. and *Niphargus* cf. *tatrensis* (with minor enchytraeid presence). The upper-left quadrant contained five wells with the community type characterized by *Bathynella natans* and *Aeolosoma* spp. Between these two community types were 12 wells representing a fourth, less clearly structured community type, distinguished primarily by the surface-dwelling copepods *Acanthocyclops venustus* and *Paracyclops imminutus*, along with various copepods, ostracods, amphipods, and annelids, including stygobionts.

4. Discussion

Although invertebrates have been recorded in 74 of the 91 wells examined in this study, representing 81.3% of the total (Dumnicka et al., 2025), which is comparable to the 81.6% colonization rate observed in 201 wells in Munich (Becher et al., 2024), crustaceans and/or annelids were detected in only about half of these wells (52%). Given the absence of significant differences in the main environmental variables between wells with and without invertebrates (Dumnicka et al., 2025), the absence of crustaceans and annelids in some wells is likely attributable to other natural factors or methodological constraints. These may include the limited dispersal capacity of these taxa in groundwater, the isolation of some aquifers, low population densities leading to non-detection during sampling, or unexamined environmental factors known to influence groundwater fauna (e.g., Marmonier et al., 2023; Hotèkpo et al., 2025), particularly in urban settings. Potential anthropogenic stressors include chemical pollution, oxygen depletion, and thermal disturbances (Becher et al., 2022). Notably, groundwater temperatures in Kraków wells were approximately 3°C higher than those in rural wells located 30–40 km from the city (Dumnicka et al., 2017, 2025).

The availability of organic matter and dissolved oxygen, largely dependent on surface-subsurface water exchange, is essential for sustaining groundwater faunal communities. In urban environments, such exchange is often impeded by extensive built-up areas, impervious surfaces, and drainage infrastructure (Becher et al., 2022). Other water chemistry parameters of Kraków urban wells, such as mineralization and nutrient levels, shaped in part by the complex geology of the region (Kleczkowski et al., 2009; Gradziński and Gradziński, 2015),



291 as well as local contamination, may also affect the faunal presence (Chowaniec et al., 2007;
292 Dumnicka et al., 2025). Similar variability in groundwater chemistry has been reported from
293 other European cities (Koch et al., 2021; Becher et al., 2022; Englisch et al., 2022; Meyer et
294 al., 2024). Unfavourable chemical conditions, such as low oxygen or high salinity, may
295 explain the absence of invertebrates in certain wells. Furthermore, the complete sealing of
296 some wells and their location in “urban desert” areas likely inhibit colonization by surface-
297 dwelling species, which can otherwise enhance local groundwater biodiversity. Given this
298 isolation, colonization pathways for surface annelids and crustaceans remain difficult to trace
299 – entry into groundwater likely occurs by chance, and populations may or may not persist.
300 Surface water proximity and rainwater infiltration probably facilitate invertebrate access. In
301 the wells studied in Kraków, a substantial proportion of the annelid and crustacean
302 communities consisted of surface-water taxa, a pattern also observed in other Polish wells
303 (Dumnicka et al., 2020; Karpowicz et al., 2021; Pocięcha et al., 2021) and those in other
304 countries (e.g., Vejdovský, 1882; Řehačková, 1953; Dalmás, 1973; Bozkurt, 2023).

305 The frequencies of annelids (19%) and crustaceans (46%) observed in this study fall
306 within the broad range reported for other large European and North African cities, where
307 annelids and crustaceans have been found in 7–58% and 3–75% of wells, respectively
308 (Vejdovský, 1882; Jaworowski, 1893; Řehačková, 1953; Vornatscher, 1972; Koch et al.,
309 2021; El Moustaine et al., 2022; Dumnicka et al., 2025).

310 We found no clear pattern of seasonal variation in the composition of crustacean and
311 annelid communities. This aligns with previous findings in the urban wells in Kraków
312 showing no significant differences in total or group-specific abundances, although taxa
313 richness and the Shannon–Wiener diversity index were significantly higher in autumn than in
314 spring (Dumnicka et al., 2025). Seasonal dynamics in invertebrate communities in urban
315 wells remain unexplored. Bozkurt (2023), for example, found only minor seasonal differences
316 in copepods, cladocerans and rotifers in 29 wells in Kilis, southern Turkey, although these
317 were not statistically tested.

318 The gamma diversity of crustaceans and annelids in Kraków wells was relatively high,
319 with 19 species recorded, including six stygobionts, although some uncertainty remains to
320 whether all juvenile *Trichodrilus* specimens represent true stygobiont species. However, the
321 species accumulation curve did not reach saturation. The low mean species frequency (2.1
322 wells), with no significant differences between stygobionts and non-stygobionts, suggests that
323 additional sampling would likely yield further species. Extrapolation metrics indicate,



324 however, that 4 to 13 additional species might be detected, implying that the current sampling
325 effort approached completeness.

326 Alpha diversity was relatively low, ranging from one to three species per well, with an
327 average of 1.4 ± 0.7 . Notably, the alpha diversity was significantly lower for the **stygobiontic**
328 species (on average < 1 species) than for surface-dwelling species (on average 1 species).
329 Similar alpha diversity (mean 1.3, range 1–2) for crustaceans and annelids has been
330 historically reported in urban wells in Lviv, Ukraine as well as in Kraków (Jaworowski,
331 1893), while more diverse communities of these invertebrate groups have also been reported
332 in wells in other cities. For instance, El Moustaine et al. (2022) documented alpha diversity
333 ranging from 1 to 6 species (mean 2.8) in eight well in Meknes, Morocco, although some taxa
334 were identified only to genus or family level. Comparative analyses remain limited due to a
335 paucity of detailed taxonomic studies in urban well fauna in Europe and the tendency of some
336 to report only higher taxonomic groups (e.g., Cyclopoida, Amphipoda, Oligochaeta) (Koch et
337 al., 2021).

338 Copepods were the most frequently encountered in Kraków wells, with six species
339 identified, **including only one stygobiont: *Diacyclops languidoides***, previously recorded in
340 Poland from five caves, a well and an interstitial habitat (Pociecha et al., 2021). Copepods are
341 key components of groundwater fauna, often comprising true stygobionts and taxa adapted to
342 subsurface habitats (Galassi et al., 2009). Previous surveys of Polish groundwater habitats
343 have reported 51 copepod species, with only four true stygobionts (Karpowicz et al., 2021;
344 Pociecha et al., 2021; Karpowicz and Smolska, 2024). In wells 37 species have been recorded
345 (including three stygobionts), primarily Cyclopoida (30 species) plus one Calanoida and six
346 Harpacticoida. Most non-**stygobiontic** copepods in our study likely originated from surface
347 waters in the Kraków area, as suggested by the presence of *Acanthocyclops venustus* and *A.*
348 *vernalis*, known from local surface habitats (see Ślusarczyk, 2003; Kur, 2012; Pociecha and
349 Bielańska-Grajner, 2015; Żurek, 2000 and Żurek et al., 2019 for surface water copepods in
350 Kraków).

351 Despite the relatively high total number of ostracod species recorded in Polish
352 groundwater environments (38 species, including nine stygobionts) and specifically from
353 wells (22 species, including eight stygobionts), only three species were found in present
354 study: one stygobiont (*Typhlocypris* cf. *eremita*) and two surface dwelling juvenile candonids.
355 *Typhlocypris eremita* is a most common representative of the stygobiontic genus
356 *Typhlocypris*, with representatives found mainly in the interstitial habitats of alluvial aquifers,
357 in the hyporheic zone along rivers and in cavernicolous habitats of central and south-eastern



Europe (Namiotko and Danielopol, 2004; Namiotko et al., 2004, 2014). In Poland *T. eremita* is a most common stygobiontic ostracod (Sywula, 1981; Pocięcha et al., 2021), occasionally collected in surface waters connected to groundwater (Namiotko, 1990; Namiotko and Sywula, 1993).

Two additional crustacean stygobionts were recorded: the bathynellacean *Bathynella natans* and the amphipod *Niphargus* cf. *tatrensis*. The former has been found in one well and five interstitial sites in southern Poland (Sywula, 1989; Pocięcha et al., 2021), while the latter is the most widespread Polish stygobiont amphipod, commonly found in caves, wells, and interstitial habitats (Dumnicka and Galas, 2017; Pocięcha et al., 2021).

Subterranean waters in Poland are known to host 111 annelid species (Dumnicka et al., 2020), with Enchytraeidae being particularly diverse, including soil-dwelling or semi-aquatic species. In Kraków, surface water oligochaetes have been rarely studied (Szarski, 1947, Dumnicka, 2002), with *Nais elinguis* being the most common taxon, mainly in lotic habitats. Previous surveys found Enchytraeidae to be relatively rare in Polish wells, with more common Tubificidae and Naididae including several stygophiles (Dumnicka et al., 2020). Similarly, our study found relatively few annelid species. Beside semi-aquatic taxa, also occasional individuals of surface-dwelling genera *Rhynchelmis* and *Aeolosoma*, were unexpectedly observed in the wells in the Kraków centre. Stygobiontic *Trichodrilus* spp. were found in four wells, potentially indicating higher water quality.

Despite low alpha diversity, beta diversity among wells was high – a pattern characteristic of groundwater ecosystems (Hahn and Fuchs, 2009; Malard et al., 2009; Stoch and Galassi, 2010; Zgamałster et al., 2014; Hose et al., 2022). Crustaceans and annelids generally occurred allopatrically, forming four main community types. One type, dominated by *Enchytraeus* gr. *buchholzi* and observed in seven wells may reflect degraded conditions according to the German groundwater ecosystem status index (GESI) (Koch et al., 2021). Another type, dominated by *Bathynella natans* with *Aeolosoma* spp. which was observed in five studied wells may indicate transitional ecological conditions, while the third type dominated in four wells by *Trichodrilus* spp. suggest relatively unaffected conditions. The forth, more heterogeneous community, distinguished primarily by the surface copepods *Acanthocyclops venustus* and *Paracyclops inminutus* is difficult to interpret ecologically. In conclusion, although species richness and abundances of annelids and crustaceans were relatively low and dominated by surface water taxa, the occurrence of six stygobiontic species in 10 of 47 wells with crustaceans and/or annelids (or of 28 wells with species-level identifications) suggests that 1/5 to 1/3 of wells in Kraków may offer relatively good



ecological conditions. Even in urban environments, groundwater fauna play a vital ecological role and may serve as bioindicators, reflecting environmental changes over multiple time scales. Accordingly, the development of a biomonitoring framework for subterranean waters, as proposed by Johns (2024), is warranted.

Conclusion

This study reveals that despite the relatively low alpha diversity of annelids and crustaceans in urban wells of Kraków, their beta and gamma diversity indicate a heterogeneous and partially natural subterranean ecosystem. The occurrence of stygobiontic species in a notable proportion of wells suggests that some groundwater habitats in the city retain ecological integrity. These findings highlight the importance of including urban groundwater fauna in biodiversity assessments and support the need for long-term biomonitoring systems to track environmental changes and protect subterranean ecosystems in urban areas.

Author Contributions

E. D.: conceptualization, investigation, methodology (collection, analysis, and interpretation of data), formal analysis, writing – original draft, writing – review and editing, project administration; J. G.: investigation, methodology (collection, analysis of data), writing – original draft, writing – review and editing, project administration; T. N.: conceptualization, investigation, methodology (analysis and interpretation of data), formal analysis, writing – original draft, writing – review and editing; A. P.: conceptualization, investigation, methodology (analysis, and interpretation of data), formal analysis, writing – original draft, writing – review and editing.

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429

430 **Conflicts of Interest**

431 The authors declare no conflicts of interest.

432

433 **Data Availability Statement**

434 The authors confirm that data supporting the findings of this study are available in the article.

435 Readers interested in other materials can request this information from the corresponding author.

436

437 **Supporting Information**

438 Additional supporting information can be found online in the Supporting Information section.

439

440 **References**

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616 **Table 1.** Occurrences of annelids and crustaceans in the studied urban wells in Kraków,
617 Poland. Stygobiontic species are shown in bold. Asterisks (*) indicate identifications based on
618 samples collected only in 2020. Addresses of the numbered wells are provided in
619 Supplementary Table S1. Complete data on water properties and the occurrences of other
620 invertebrate groups are available in Dumnicka et al. (2025).
621

Taxon	Number of individuals	Wells
Annelida		
<i>Aeolosoma hyalinum</i> Bunke, 1967	2	3
<i>Aeolosoma</i> spp.	4	21, 73, 82
<i>Achaeta</i> sp. juv.	1	35
<i>Enchytraeus</i> gr. <i>buchholzi</i> Vejdovský, 1879	3	61, 68
Enchytraeidae gen. sp. juv. (mainly <i>Enchytraeus</i>)	8	18, 29, 32, 44, 55, 56, 68, 77, 82
<i>Marionina argentea</i> (Michaelsen, 1889)	1	3
<i>Rhynchelmis</i> sp. juv.	1	57
<i>Trichodrilus cernosvitovi</i> Hrabě, 1938	1	28
<i>Trichodrilus</i> spp. juv.	11	12, 28, 29, 55
Oligochaeta gen. spp. juv.	2	18, 35
Crustacea Ostracoda		
<i>Pseudocandona</i> sp. juv.	61	19, 48
<i>Typhlocypris</i> cf. <i>eremita</i> (Vejdovský, 1882)	5	48
Candonidae gen. sp. juv.	1	18
Crustacea Copepoda		
<i>Acanthocyclops robustus</i> (Sars, 1863) *	6	84
<i>Acanthocyclops venustus</i> (Norman & Scott, 1906) *	9	69, 84, 85
<i>Acanthocyclops vernalis</i> (Fischer, 1853) *	108	84
<i>Diacyclops crassicaudis</i> (Sars, 1863) *	3	83
<i>Diacyclops languidoides</i> (Lilljeborg, 1901) *	4	84a
<i>Paracyclops imminutus</i> Kiefer, 1929 *	14	86, 91
Cyclopoida copepodites *	9	69, 82, 84, 85, 86, 88, 91
Cyclopoida nauplii *	4	84, 91
Copepoda not identified to species	ca. 500	2, 3, 6, 9, 12, 21, 23, 25, 26, 27, 29, 31, 32, 35, 37, 40, 48, 49, 50, 52, 56, 57, 58, 59, 60, 61, 62
Crustacea Bathynellacea		
<i>Bathynella natans</i> Vejdovský, 1882	38	64, 73, 75
Crustacea Amphipoda		
<i>Niphargus</i> cf. <i>tatrensis</i> Wrześniowski, 1888	7	48, 50

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624 **Table 2.** Estimated total number of species predicted by four extrapolation estimators based
625 on abundance data for 19 crustacean and annelid species from 182 samples collected in 91
626 urban wells in Kraków. SD = standard deviation.

627

Estimator	Expected species richness
Chao 1 \pm SD	31.5 \pm 17.1
Jackknife 1	28.9
Bootstrap	23.2
Michaelis-Menten	31.4

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Table 3. Diversity measures for crustaceans and annelids found in the 28 out of 91 studied urban wells in Kraków, shown for the full dataset and separately for **stygobiontic** and non-**stygobiontic** species subsets. Gamma diversity = total species richness, Beta diversity = global Whittaker species turnover index, Alpha diversity = average species richness per well, Species frequency = number of wells in which species occurred. SD = standard deviation, BCa = bias-corrected and accelerated bootstrap method. Differences in mean alpha diversity and species frequency between stygobiontic and non-stygobiontic datasets were tested using one-way permutational analysis of variance PERMANOVA: F = permutation-based test statistic, P = probability based on 9,999 permutations. Statistically significant value is bolded.

	Full species dataset	Stygobiontic species subset	Non-stygobiontic species subset	Statistical difference
Gamma diversity	19	6	13	
Beta diversity	12.3	13.0	12.0	
Alpha diversity				
Range	1–3	0–2	0–3	
Mean	1.43	0.43	1.00	F = 4.874 P = 0.026
SD	0.69	0.69	0.67	
BCa 95% Lower limit	1.18	0.14	0.71	
BCa 95% Upper limit	1.64	0.64	1.21	
Species frequency				
Range	1–10	1–4	1–10	
Mean	2.11	2.00	2.15	F = 0.020 P = 0.953
SD	2.13	1.26	2.48	
BCa 95% Lower limit	1.26	1.00	1.08	
BCa 95% Upper limit	3.05	2.83	3.38	



Table 4. Pearson correlation coefficients between the first two axes of Principal Coordinate Analysis (PCoA) and relative abundance of eight crustacean and annelid species showing correlation values > 0.2 with at least one axis. Stygobiontic species are shown in bold.

Species	PCoA1	PCoA2
<i>Enchytraeus</i> spp.	0.979	0.061
<i>Trichodrilus</i> sp. juv.	-0.130	-0.903
<i>Bathynella natans</i>	-0.303	0.419
<i>Trichodrilus cernosvitovi</i>	-0.130	-0.412
<i>Aeolosoma</i> spp.	-0.090	0.339
<i>Acanthocyclops venustus</i>	-0.210	0.085
<i>Paracyclops imminutus</i>	-0.203	0.080
Candonidae juv.	0.247	0.021



651 **Captions of Figures**

652

653 **Fig. 1.** Map of the study area showing the locations of urban wells sampled within the
654 Kraków metropolitan area for the study of annelids and crustaceans (modified from
655 Dumnicka et al. 2025). Wells where stygobionts were detected are marked as follows: B =
656 *Bathynella natans*, D = *Diacyclops languidoides*, N = *Niphargus* cf. *tatrensis*, T =
657 *Typhlocypris* cf. *eremita*, Tr = *Trichodrilus* spp. juv. + *T. cernosvitovi*.

658

659 **Fig. 2.** Photographs of representative surveyed wells: left – bored/dug well with piston pump;
660 right – artesian deep well with tap.

661

662 **Fig. 3.** Boxplots summarizing the chemical and physical characteristics of groundwater in the
663 studied urban wells in Kraków, Poland. Boxes represent the mean \pm standard deviation, with
664 whiskers indicating full range (minimum to maximum) of values.

665

666 **Fig. 4.** Mean cumulative species richness of the 19 studied invertebrate species (including
667 stygobiontic and non-stygobiontic annelids, ostracods, copepods, bathynellaceans, and
668 amphipods) plotted against the number of 182 samples from 91 urban wells in Kraków.
669 Whiskers represent ± 1 standard deviation.

670

671 **Fig. 5.** Non-metric Multidimensional Scaling (nMDS) ordination plot showing no significant
672 differences in annelid and crustacean community structure between groundwater samples
673 collected in spring (light green circles) and autumn (brown diamonds) from the studied urban
674 wells in Kraków, Poland.

675

676 **Fig. 6.** Principal Coordinate Analysis (PCoA) of annelid and crustacean community
677 composition in groundwater from the studied urban wells in Kraków, Poland. Points represent
678 individual wells (well numbers correspond to Table 1 and Supplementary Table 1). Pearson
679 correlation vectors with values > 0.2 on at least one axis are overlaid.

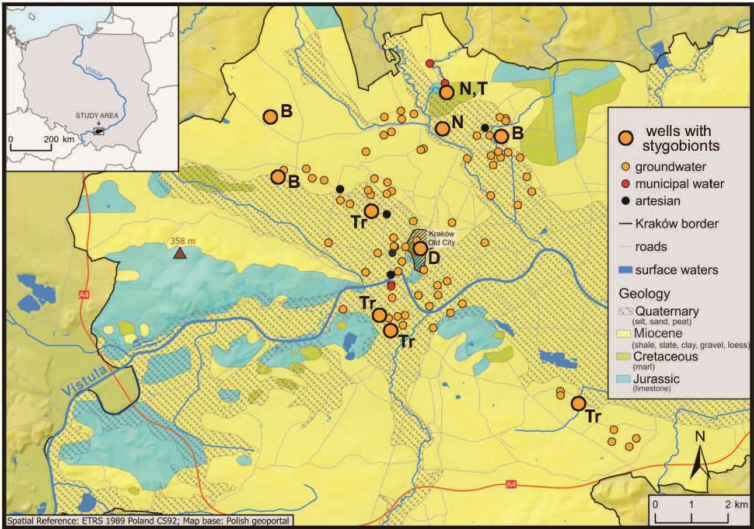
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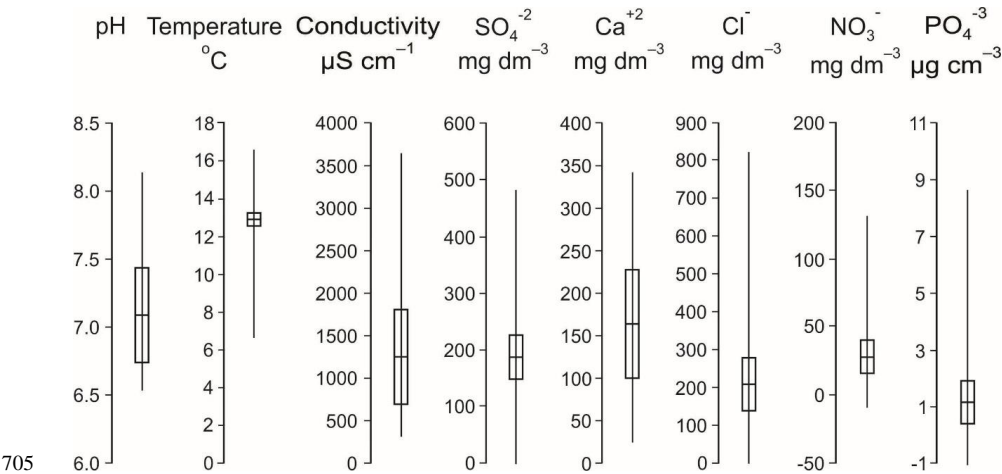
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Fig. 2. Photographs of representative surveyed wells: left – bored/dug well with piston pump; right – artesian deep well with tap.



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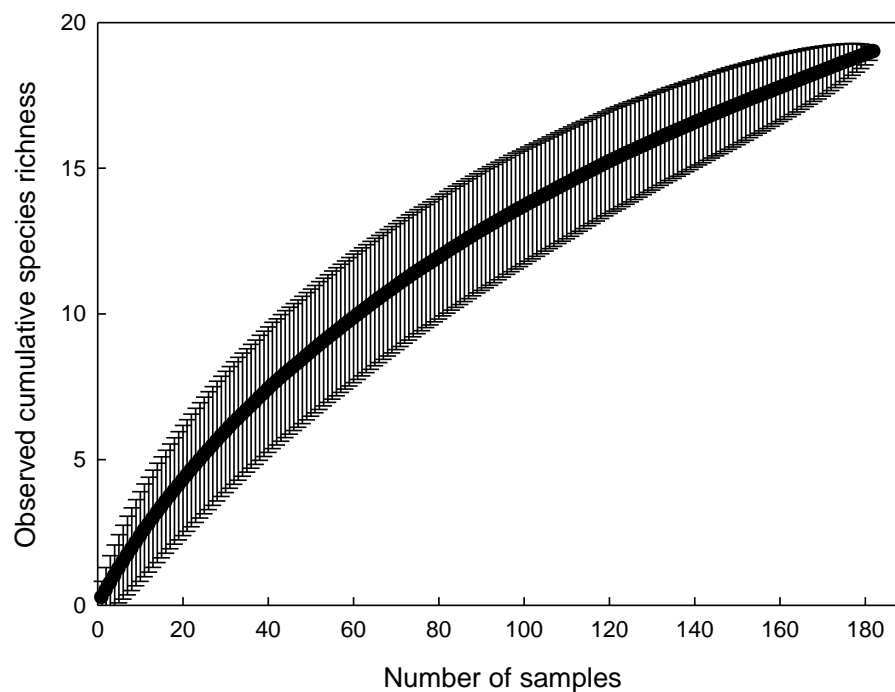


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719 stygobiontic and non-stygobiontic annelids, ostracods, copepods, bathynellaceans, and
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721 Whiskers represent ± 1 standard deviation.

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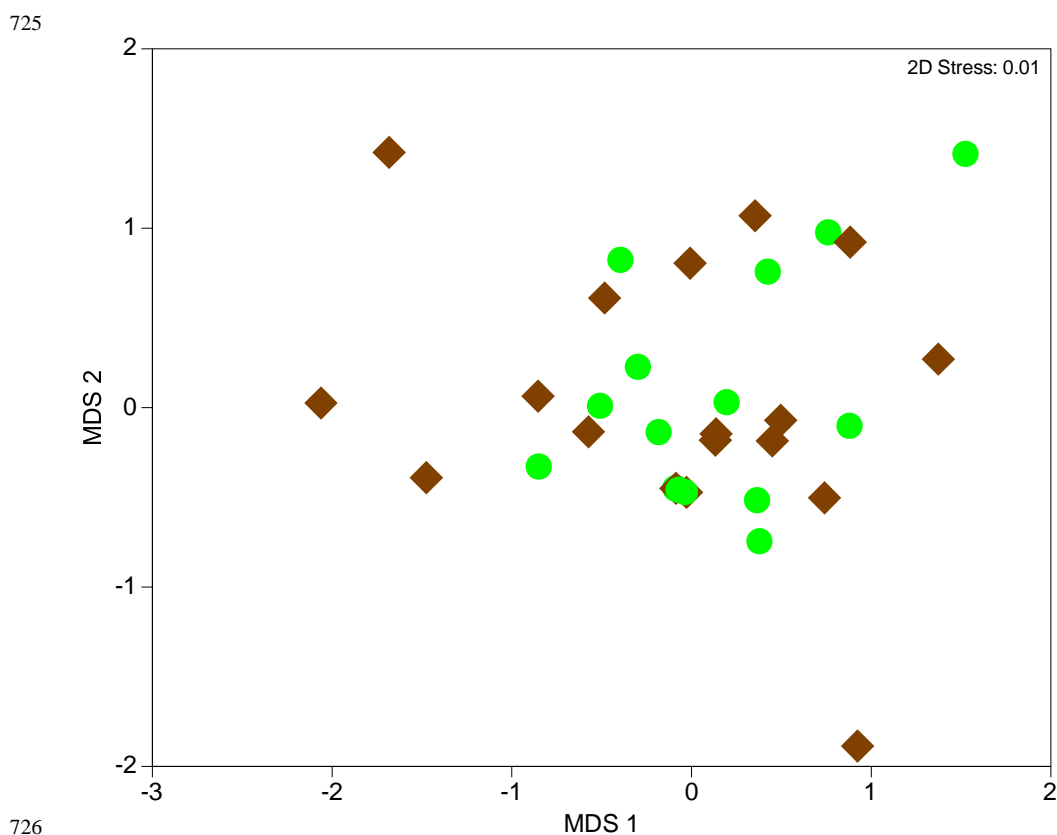


Fig. 5. Non-metric Multidimensional Scaling (nMDS) ordination plot showing no significant differences in annelid and crustacean community structure between groundwater samples collected in spring (light green circles) and autumn (brown diamonds) from the studied urban wells in Kraków, Poland.

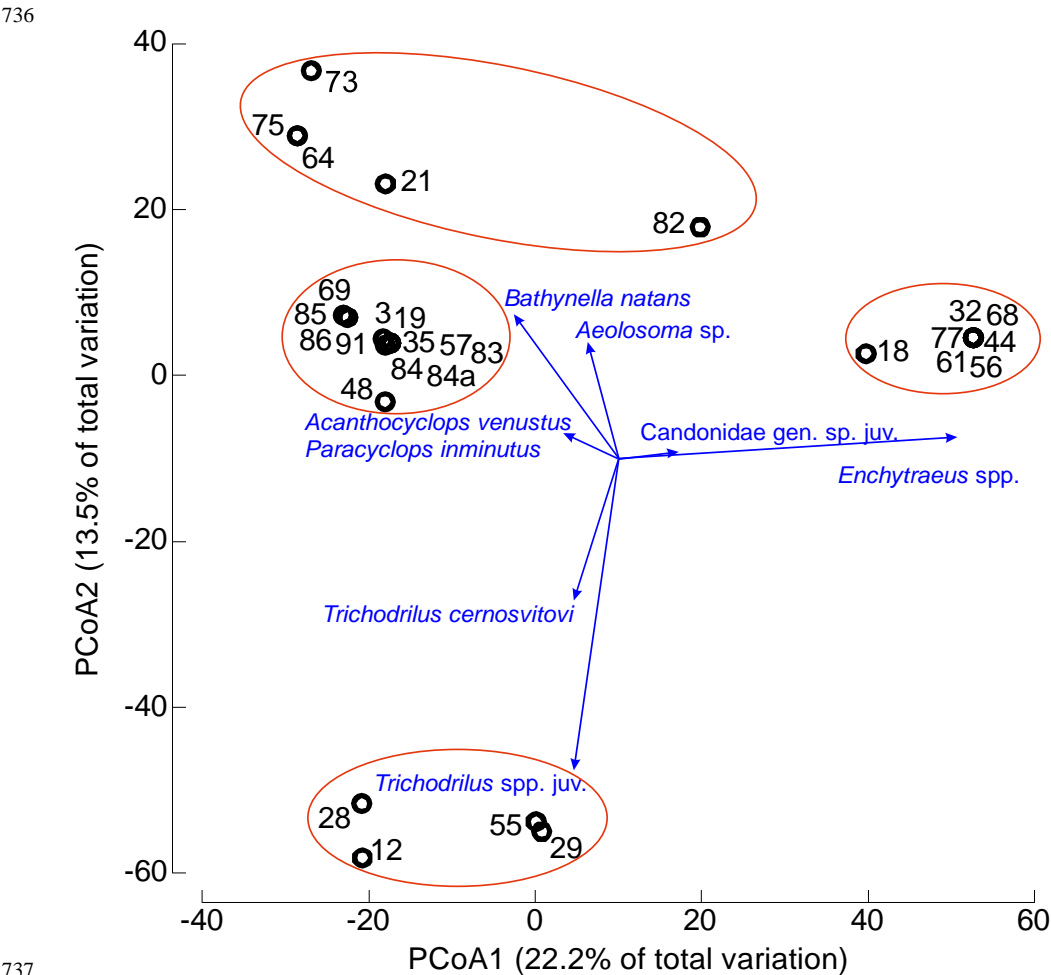


Fig. 6. Principal Coordinate Analysis (PCoA) of annelid and crustacean community composition in groundwater from the studied urban wells in Kraków, Poland. Points represent individual wells (well numbers correspond to Table 1 and Supplementary Table 1). Pearson correlation vectors with values > 0.2 on at least one axis are overlaid.