Accepted version: Ishak S., Ghannem S., Alotaibi R.M., Alkatheri R.S., Alharbi M.F., Almutrif A.M., Grassi E. Semprucci F., Badraoui R., Dilara S., Hamadi N.B., Khezami L., Rudayni H.A., Boufahja F., 2025. Effects of antibiotics and metals on meiofauna assessed through taxon/functional and modeling tools: a case study of amoxicillin and copper, Marine Pollution Bulletin, 216, 18022. https://doi.org/10.1016/j.marpolbul.2025.118022.

Effects of antibiotics and metals on meiofauna assessed through taxon/functional and modeling tools: a case study of amoxicillin and copper

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ABSTRACT

This research examines the impacts of amoxicillin and copper, separately and together, on the taxonomic and functional diversity of free-living marine nematodes. Sediment samples were gathered from the Jeddah shoreline in Saudi Arabia, and meiobenthic organisms were subjected to two concentrations of amoxicillin [550 and 1100 ng/L] and copper [130 mg/kg dry weight (dw) and 260 mg/kg dw] in microcosms for 30 days. The findings indicated a higher nematode tolerance than that of polychaetes, copepods, isopods, amphipods, and cumaceans. A notable decrease in both nematode species abundance and diversity was observed as contaminant levels rose, resulting in the reduction of sensitive bioindicators, specifically *Paracanthonchus sadspitensis*, *Dorylaimopsis timmi*, *Cinctonema papillata*, *Eleutherolaimus obtusicaudatus*, *Terschellingia longicaudata*, *Theristus poloris*, *Halalaimus longicaudatus*, *Parodontophora breviseta*, and *Theristus pertenuis* for copper, as well as *C. papillata*, *T. longicaudata*, *H. longicaudatus*, *T. pertenuis*, *D. timmi*, and *Viscosia viscosia* for amoxicillin. Conversely, tolerant/opportunistic species such as *Metoncholaimus albidus* for amoxicillin and *Daptonema oxycerca* for both copper and amoxicillin showed an increase in abundance. The pairing of amoxicillin and copper demonstrated a synergistic or additive toxic impact. Furthermore, pollution changed the functional characteristics of nematodes, leading to a rise in detritivore

65 species with clavate tails, and a decline in microvore species with conical and filiform tails. A
66 computational analysis also supported these findings by evaluating the toxicokinetics and
67 molecular interactions of amoxicillin and copper.

68

69 **Keywords:** Amoxicillin; Copper; Meiofauna, Taxonomic structure, Functional traits ;
70 Modeling

71

72 **1. Introduction**

73

74 The use of antibiotics, their presence in the environment, and the spread of antimicrobial
75 resistance represent a significant global health issue (Louati, 2013; Avio et al., 2015; Carvalho
76 and Santos, 2016). Antibiotics can be natural, synthetic, or semi-synthetic and possess the
77 ability to kill or inhibit the growth of microorganisms (Kummerer, 2009a). These compounds
78 are biologically active agents with antibacterial, antifungal, and antiparasitic properties,
79 designed as medications to treat bacterial infections in both humans and animals and also used
80 as food additives or for disease prevention in livestock (Kummerer, 2009b). The use of
81 pharmaceutical products in this therapeutic category has increased dramatically in recent years,
82 driven by population growth and rising demand for animal proteins, necessitating higher
83 production through growth factors and antibiotics (Zhao et al., 2019; Van Boeckel et al., 2015).
84 Consequently, antibiotics ingested by humans or animals are metabolized and excreted in
85 various active chemical forms through urine and feces into urban wastewater (Kovalakova et
86 al., 2020).

Antibiotic residues are also frequently detected in water bodies such as river water and irrigation dam water and effluents from hospitals and sewage treatment facilities (Monteiro and Boxall, 2010; Watkinson et al., 2009 ; Abera et al., 2025). They were interestingly found in biological matrices such as cow urine (Abera et al., 2025) and food such as milk, lettuce, carrot, and tomato (Lakew et al., 2023 ; Abera et al., 2025). The highest levels of antibiotic residues found in wastewater typically range in milligrams per liter, potentially exceeding 1000 mg/L in industrial areas in China and India (Larsson, 2014; O'Neill et al., 2015). Wastewater from hospitals can contain small amounts of antibiotics, around a few milligrams per liter (Watkinson et al., 2009). In the particular case of amoxicillin, wastewater treatment plants frequently fall short in removing residues of pharmaceutical compounds, leading to its presence in ocean ecosystems (Kushneet et al., 2021). Although amoxicillin has relatively low acute and chronic toxicity on aquatic life forms such as *Daphnia magna*, and fish, it may still pose ecological threats because of possible bioaccumulation (Sodhi et al., 2021).

It is recognized that when medications are introduced into aquatic ecosystems, they can interact with other substances, including metals, possibly changing the physiology of non-target marine and benthic species (Osorio et al., 2016). Copper is a versatile metal, with a global demand in 2050 relative to 2010 likely to increase by 140% (de Koning et al., 2018; Watari et al., 2021). It was found in various matrices, including soft drinks (Fanta and Sprite), food supplements (commercially sold multi-mineral/vitamin tablets), and environmental water (tap water and wastewater) (Tesfaye et al., 2022). Its levels in marine sediments are affected by industrial operations, antifouling substances, and sediment characteristics, presenting ecological hazards to marine organisms, with differing toxicity and bioavailability based on sediment traits and copper forms (Yang et al., 2018). These investigations indicate that elevated copper levels in water bodies lead to a notable change in prokaryotic community structure,

impacting biodiversity and community composition, with *D. similis* and *R. subcapitata* being the most susceptible (Oliveira-Filho et al., 2004).

Utilizing bioindicators—organisms that facilitate the evaluation of ecosystem health—allows researchers to assess the impact of pollution on the well-being and composition of living organisms before a significant disruption occurs in the entire biota and ecosystem (Baldrighi et al., 2019, 2021 ; Leasi et al., 2021). Meiobenthic taxa are considered bioindicators of ecosystem health, especially free-living marine nematodes (Gambi et al., 2020 ; Leasi et al., 2021 ; Semprucci et al., 2021). These worms make up the most diverse and populous group (60-90 %) of global meiofauna and are also the most varied group inhabiting marine sediments (Ape et al., 2018 ; Armenteros et al., 2019 ; Schratzberger and Somerfield, 2020; Cocozza di Montanara et al., 2022). Interestingly, few studies indicate that the quantitative and qualitative features of meiobenthic nematodes are significantly influenced by the presence of copper (Hedfi et al., 2008) and the antibiotics penicillin G and ciprofloxacin in their immediate environment (Nasri et al., 2015; 2024). Although copper's impact on free-living nematodes has been partially researched at the taxonomic level (Hedfi et al., 2008), its influence on other meiobenthic groups, its interaction with amoxicillin, and amoxicillin's effect on meiobenthic organisms are entirely unexamined.

The topic investigated in this research has recently emerged as a significant global concern. Thus, this research intends to address primarily the gap concerning amoxicillin, whose impacts have yet to be investigated on the major meiobenthic group, nematodes. The inquiries explored were: (1) Does amoxicillin negatively affect traits of meiobenthic nematodes? and (2) If so, what interactions occur with copper? (3) Do nematodes exhibit taxon/functional changes after their exposure to amoxicillin, copper, and their combinations? The applied experimental approach was supported by several computational methods related to amoxicillin and Germ-Line Development protein 3 (GLD-3) as well as the Sex-Determining Protein (SDP).

2. Materials and methods

2.1. Sampling and acclimatization phase

Sediment samples were collected on March 17, 2023, from an undisturbed coastal location (21°14'0.07"N, 39°8'43.13"E) situated in the upper sublittoral zone along the Jeddah coast, Saudi Arabia. Samples were obtained from the top 5 cm of the sediment layer, at a depth of 50 cm in the water column (Hedfi et al., 2008), using Plexiglas hand cores with an internal diameter of 3.6 cm and a cross-sectional area of 10 cm^2. This layer serves as a microhabitat that exhibits the greatest taxonomic richness and abundance of meiofauna (Bin-Jumah, 2024).

On the sampling day, four abiotic factors were assessed at the sediment-water interface: temperature and salinity were recorded with a Thermo-Salinometer (model WTW LF 196) from German Wilhelm, while dissolved oxygen levels were measured using an Oxymeter (model WTW OXI 330/Set), also from German Wilhelm. A pH meter (model WTW pH 330 / SET-1) was employed to assess pH values. In the lab, the moisture level was ascertained by drying the gathered sediment at 45°C until a constant weight was reached (Boufahja et al., 2011). Subsequently, the sediment was passed through 63 µm sieves with a water jet and then dried again at 45°C to analyze the sediment's grain fractions (Buchanan, 1971). Cumulative curves were developed to evaluate the mean grain size, as observed by Buchanan (1971), while the mass loss method at 450°C for 6 hours was used to ascertain the total organic matter content (Fabiano and Danovaro, 1994).

2.2. Selected concentrations of amoxicillin and copper

159 The concentrations of copper in marine sediments ranged from a baseline of 30-40
160 mg/kg to a peak concentration of 1200 mg/kg (Morrisey et al., 1996; Encina-Montoya et al.,
161 2024). Additionally, standard copper concentrations vary between 4.5 and 123 mg/kg in the
162 deeper sediment layers (> 10 cm) of the ocean floor (Environment Canada, 1998). Copper
163 toxicity in marine sediments has been reported at 258 mg/kg for amphipods (Parks et al., 2018),
164 700 mg/kg for meiobenthic nematodes (Hedfi et al., 2008), and 708 mg/kg for mysids (Parks et
165 al., 2018). Two concentrations were ultimately selected for copper, 130 and 260 mg/kg dry
166 weight (dw). The lower concentration is roughly equivalent to the maximum level detected in
167 deeper, relatively unpolluted layers (>10 cm, 123 mg/kg). The highest concentration was
168 determined based on the lowest ecotoxicological level that causes toxicity in aquatic organisms
169 (258 mg/kg).

170 Many acute toxicity studies have investigated the impacts of amoxicillin on different
171 marine biological models. Nonetheless, many of them involved much shorter durations
172 compared to the typical timeframe used for meiofauna (i.e., 30 days). For instance, amoxicillin
173 exhibits a 15-minute acute toxicity level of 56.23 mg/L for the bacterium *Vibrio fischeri*
174 (Sönmez and Sivri, 2020). As an illustration, Lützhøft et al. (1999) found an EC50 of 0.0037
175 mg/L for the alga *Microcystis aeruginosa* during a 7-day exposure duration. In comparison,
176 Park and Choi (2008), along with Lee et al. (2021), reported a 48-hour EC50 value for the
177 cladocera *D. magna* that was greater than 1000 mg/L. To choose concentrations for the current
178 experiment, it was preferred to consult the environmental concentrations. Amoxicillin can be
179 detected in aquatic environments at low levels (from nanograms to micrograms per liter) (Sodhi
180 et al., 2021). For example, amoxicillin concentrations were detected in various surface water
181 bodies in the United Kingdom, Australia, and Spain, ranging from 39 to 245 ng/L (Watkinson
182 et al., 2009; Kazprzyk-Horden et al., 2007; Fatta-Kassinos et al., 2011). The presence of this
183 antibiotic was more noticeable in raw urban sewage, wastewater treatment plant influents, or

hospital effluents across Australia, Italy, and Spain, with levels ranging from 622 to 1670 ng/L (Watkinson et al., 2009; Zuccato et al., 2010). Based on these data, two concentrations were selected: 550 and 1100 ng/L. The lower concentration may represent a relatively minor contamination level, as it is roughly twice the maximum environmental concentration under normal circumstances (245 ng/L). The higher concentration was roughly equivalent to the average within the range of 622-1670 ng/L, indicating moderate amoxicillin contamination.

2.3. Contamination of water and sediment

Amoxicillin trihydrate (Sigma Aldrich, CAS Number: 61336-70-7) was initially dissolved in pre-filtered seawater (filtered using 0.7 µm pore-size Glass Microfibre filters, GF/F, Whatman) without solvents, to create a 1 g/L stock solution, which was then stored in dark at -20°C (de Moraes et al., 2022) until utilized for contaminating the seawater within microcosms. Copper chloride dihydrate stock solutions (Sigma Aldrich, CAS Number: 10125-13-0) were also prepared using the same prefiltered (0.7 µm pore-size Glass Microfibre GF/F, Whatman) seawater. In the lab, the sediment designated for contamination underwent three cycles of freezing at -20°C for 12 hours, followed by thawing at room temperature for 48 hours to remove all life forms (Schratzberger et al., 2004). The sediment was subsequently passed through a 63 µm mesh to eliminate larger particles (Boufahja et al., 2015). The copper was thoroughly mixed into the sediment using a food mixer and left to equilibrate for one week at 4°C in the dark (Bin-Jumah, 2023). After this acclimatation period, 100 g samples of dry sediment were pre-contaminated with appropriate amounts of copper and combined with 200 g of natural sediment (containing live meiofauna) to reach final copper concentrations of 130 and 260 mg/g dw. In the control group, 100 g of azoic, uncontaminated sediment was mixed with 200 g of natural sediment.

208

209 *2.4. Experiment set-up*

210 The microcosms consisted of 1.5 L glass bottles (Boufahja et al., 2015), following the
211 framework followed by Badraoui et al. (2023). Each bottle contained 300 g of sediment and 1
212 liter of pre-filtered seawater (filtered through 0.7 µm pore-size Glass Microfibre filters, GF/F,
213 Whatman) obtained from the collection site (Leasi et al., 2021). These microcosms functioned
214 as closed systems, with continuous aeration throughout the experimental period provided by
215 aquarium pumps (Nasri et al., 2020).

216 A total of nine treatments were established, including one control group; two treatments
217 with copper (Cu1 = 130 mg/kg dw and Cu2 = 260 mg/kg dw), two treatments with amoxicillin
218 (AMX1 = 550 ng/L and AMX2 = 1100 ng/L), and four combinations of different treatments
219 (AMX1/Cu1, AMX2/Cu2, AMX2/Cu1, and AMX1/Cu2). In total, 27 microcosms were set up
220 at 4°C in the absence of light, with three replicates for each treatment.

221

222 *2.5. Meiofauna study*

223 Sediment samples were preserved in a 4% buffered formaldehyde solution, with a few
224 drops of a Rose Bengal solution (0.2 g/L) added to stain meiobenthic organisms pink,
225 facilitating their distinction from inert substrate particles (Waweru et al., 2024). Meiofauna was
226 subsequently extracted from the sediments using the classic
227 levigation/resuspension/decantation method, and the biota was retained on a 40 µm sieve
228 (Rzeznik-Orignac et al., 2017). Nematode counting was performed using a 50x
229 stereomicroscope (Wild Heerbrugg model M5A), and the specimens were immersed in 10%
230 glycerol, gradually concentrated in anhydrous glycerol, and then mounted on permanent slides

(Bin-Jumah, 2023). Identification of genus and species was performed using a Nikon DS-Fi2 camera connected to a Nikon microscope, with NIS Elements Analysis Software (Version 4.0, Nikon 4.00.07–build 787–64 bit). Pictorial keys by Platt and Warwick (1988) and descriptions from the NeMys database (Nemys, 2024) were used for genus and species identification, respectively.

For the analysis of functional traits, two nematode features were assessed: tail shape and feeding group. Trophic groups, defined by the structure of the oral cavity, are divided into four categories: epistrate grazers (2A), deposit-selective feeders (1A), non-selective feeders (1B), and omnivorous predators (2B) (Wieser, 1953). Tail shape was also categorized into four types: conical (co), clavate (cla), short/round (s/r), and elongated/filiform (e/f) (Semprucci et al., 2018).

2.5. Computational modeling

This study involved examining the binding scores and molecular interactions of amoxicillin with two receptors from the nematode model *Caenorhabditis elegans*, which serves as a representative species for nematodes. The 3D crystal structures of GLD-3 and SDP were obtained from the RCSB Protein Data Bank. GLD-3 and SDP were prepared for docking by removing crystallographic water molecules, incorporating polar hydrogens, and adding Kollman charges, as previously described (Ben Saad et al., 2023; Boudjema et al., 2024). Using the CHARMM force field, we analyzed the interactions between amoxicillin and the receptors GLD-3 and SDP to create ligand-receptor complexes, following methodologies outlined in prior studies (Badraoui et al., 2023; Ishak et al., 2024; Rahmouni et al., 2024). The resulting molecular interactions were recorded and characterized (Ben Saad et al., 2023; Elyousfi et al., 2024; Ishak et al., 2024). We selected GLD-3 and SDP for this analysis due to their critical roles

in germ lineage development and sex determination in nematodes, particularly in *C. elegans* (Badraoui et al., 2023; Ishak et al., 2024).

2.7. Data processing

In this study, various biodiversity indices were assessed for free-living marine nematodes using PRIMER version 5.0 software. The faunal paramenters comprised total abundance, species number, Margalef's species richness, and the Shannon-Wiener index (H'*e*). One-way ANOVA alongside Tukey's HSD tests was employed to compare the treatments. The Chi-square test was employed to evaluate variations in relative abundances among different functional groups after √-transformation. Three multivariate analyses were conducted. Non-metric multidimensional scaling (nMDS) diagrams were generated based on both species abundance and functional traits, applying square root transformations and calculating Bray-Curtis similarity coefficients (Bray and Curtis, 1957). The analysis of similarity (ANOSIM) was used to evaluate changes in the taxonomic and functional composition of nematode groups among the different treatments. In addition, similarity percentages (SIMPER) analysis was carried out to determine the contribution of individual species or functional groups to the observed dissimilarities among treatments (Clarke, 1993).

3. Results

3.1. Abiotic traits of the collection site

The standard depth for collecting sediment was 50 cm. The measured temperature was 34.6 ºC, whereas the dissolved oxygen concentration was 9.21 mg/l. Furthermore, it was observed that the recorded salinity and pH levels were 41.45 PSU and 8.4, respectively. The gathered sediment had 9.62 ± 0.71% silt/clay particles (smaller than 63 µm) and 90.38 ± 0.71% coarse particles (larger than 63 µm). The sandy fraction showed a median particle size of 283 ± 22 µm, a moisture level of 19.63 ± 2.17%, and an organic matter content of 7.04 ± 0.92%

3.2. Abundance of meiobenthic organisms

Nematodes, along with the next five dominant groups (polychaetes, copepods, isopods, amphipods, and cumaceans), constituted the meiobenthos collected from the sampling site (see Table 1). Nonetheless, since the nematofauna was the primary focus of the present experiment, more detailed analyses were restricted to this phylum. Table 1 illustrates the changes in nematode abundance following exposure to amoxicillin and/or copper. The total nematode abundance was significantly lower in all treatments compared to the control (Tukey-HSD test: p-values < 0.05), with the most pronounced reductions observed in AMX2/Cu2, AMX2, and AMX2/Cu1.

Furthermore, pairwise comparisons of abundance between combined treatments (AMX2/Cu2 and AMX1/Cu1) and single treatments (Cu1, Cu2, AMX1, and AMX2) indicated that combined exposures had a more substantial impact than the individual application of copper and amoxicillin (see Table 1). A similar trend was observed for the other meiobenthic groups, whose populations also declined—in many cases more markedly than nematodes (Tukey HSD test, Table 1). Notably, a complete absence of certain taxa occurred in 2 to 6 out of the 8 treatments involving copper and amoxicillin. The most affected group was amphipods,

299 which were absent following exposure to AMX1, AMX2, AMX1/Cu1, AMX2/Cu2,
300 AMX2/Cu1, and AMX1/Cu2.

301

302 *3.3. Taxonomic diversity of meiobenthic nematodes*

303 The nematode community comprised 25 species belonging to 5 orders and 13 families
304 (Table 2). The dominant order was Monhysterida, with Xyalidae being the most represented
305 family. After the experiment, the species *Eleutherolaimus obtusicaudatus* was the most
306 abundant in the control assemblage, accounting for 8.59 ± 0.78% of the total community. This
307 species was eliminated in certain treatments (AMX2/Cu2), while in others (AMX1/Cu2), its
308 abundance increased relative to the control (see Table 3). Similarly, *Cinctonema papillata* was
309 prevalent in the control group, comprising 7.27 ± 2.98%; but was absent in the AMX2 and
310 AMX2/Cu1 treatments and exhibited minimal presence in AMX2/Cu2 (0.52 ± 0.91%) (Table
311 3).

312 It is noteworthy that some species present in the control were completely eliminated at
313 higher concentrations and in combined treatments. These include *Daptonema oxycerca*
314 (AMX2/Cu2 and AMX2/Cu1), *Halalaimus longicaudatus* (AMX1), *Metoncholaimus albidus*
315 (AMX1/Cu2 and AMX2/Cu1), *Terschellingia communis* (AMX2/Cu2 and AMX2/Cu1), and *T.*
316 *longicaudata* (AMX1/Cu1 and AMX2/Cu2). Conversely, certain species initially found in low
317 abundance in the control became prevalent at higher concentrations and under combined
318 exposures. Notable examples include *Desmodora pontica* in treatments AMX2/Cu2 and
319 AMX2/Cu1, and *M. albidus*, in AMX2/Cu2 (refer to Table 3).

320 Figure 1 illustrates a significant reduction in taxonomic richness in the AMX2/Cu2,
321 AMX2, AMX2/Cu1, and AMX1/Cu2 treatments (Tukey's HSD test: $p < 0.0001$). For other
322 diversity indices, notable differences (Tukey's HSD test) were noted, including Margalef's (*p*-

323 value = 0.031) and the Shannon-Wiener (*p*-value = 0.046) indices, between AMX2/Cu2 and the
324 control (see Fig. 1).

325 Figure 2 presents the clustering analysis and nMDS ordination, demonstrating the
326 impact of AMX and Cu contamination on species distribution (Stress = 0.12). As a result, three
327 distinct groups were identified: (1) Group 1, consisting solely of AMX2/Cu2, represents the
328 assemblages most dissimilar to the control nematofauna; (2) Group 2, represented by
329 AMX2/Cu1 and AMX2, includes assemblages closest to the control replicates; and (3) Group
330 3 comprises the control assemblage along with the remaining treatments**.**

331 As shown in Table 3, the SIMPER analysis indicated that nine species
332 (*Paracanthonchus sadspitensis*, *Dorylaimopsis timmi*, *C. papillata*, *E. obtusicaudatus*, *T.*
333 *longicaudata*, *Theristus poloris*, *H. longicaudatus*, *Parodontophora beviseta*, and *Theristus*
334 *pertenuis*) were the primary contributors to the dissimilarity observed between control and
335 copper treatments; all of these species exhibited a decline in abundance.

336 Similarly, six species showed reduced abundance after exposure to amoxicillin
337 compared to the controls: *C. papillata*, *T. longicaudata*, *H. longicaudatus*, *T. pertenuis*, *D.*
338 *timmi*, and *Viscosia viscosia*. Conversely, certain species showed increased abundance under
339 specific treatments. In the case of copper, *D. oxycerca* increased in abundance, while under
340 the lowest amoxicillin concentration (AMX1), *T. communis*, *D. oxycerca*, *M. albidus*, and
341 *Ptycholaimellus sindhicus* were more abundant.

342 The degree of taxonomic changes varied among treatments (see Table 4). The highest
343 dissimilarity values compared to the controls were observed in AMX2/Cu2 (54.53%),
344 AMX2/Cu1 (41.04%), and AMX2 (39.33%). All other treatments resulted in 19.37% to 29.18%
345 dissimilarity compared to the uncontaminated control (UC). Finally, it was observed that

346 increasing concentrations of both copper and amoxicillin were associated with higher average
347 dissimilarity values.

348

349 *3.4. Functional traits of meiobenthic nematodes*

350 Following the experiment, nematodes from groups 1B and 1A were the most abundant
351 in the control samples, accounting for 49.69 ± 1.38% and 19.12 ± 2.22% of the total
352 nematofauna, respectively. Significant changes were observed as a result of contamination by
353 amoxicillin, copper, or their combinations (refer to Fig. 3). Cluster analysis combined with the
354 nMDS ordination revealed a separation into three categories: Group 1, consisting solely of the
355 AMX2/Cu2 treatment, was the most distinct from the control assemblage, Group 2, which
356 included Cu1, Cu2, UC, AMX1, AMX1/Cu2, AMX1, and AMX1/Cu1; and Group 3, composed
357 of AMX2/Cu1 and AMX2, which occupied an intermediate position on the ordination plot.

358 Most treatments resulted in a decrease in the abundance of group 1A, as shown by the
359 SIMPER analysis (see Table 5 and Fig. 3). This decline was responsible for the differences
360 observed in 6 of the 8 treatments (Cu1, Cu2, AMX1, AMX2, AMX1/Cu1, and AMX1/Cu2)
361 relative to the controls. Conversely, the trophic shifts in five treatments (Cu1, AMX2,
362 AMX2/Cu2, AMX1/Cu2, and AMX2/Cu1) showed an increase in the proportion of group 2B.
363 To a lesser extent, increases in trophic group 1B also contributed to the trophic differences in
364 three treatments: Cu2, AMX1, and AMX2/Cu2.

365 The extent of trophic variation differed among treatments (refer to Table 4). The highest
366 values compared to the controls were noted for AMX2/Cu2 (63.26%), AMX2 (26.54%), and
367 AMX2/Cu1 (26.54%). All remaining treatments resulted in only 8.79 to 19.90% taxonomic
368 variation relative to UC. As in previous observations, increasing concentration was associated
369 with higher average dissimilarity values for both copper and amoxicillin (refer to Table 4).

370 Nematodes with conical and clavate tails were the most prevalent in the control group,
371 with relative abundances of 47.02 ± 3.53% and 37.65 ± 1.79%, respectively. Cluster analysis
372 and the nMDS ordination (Fig. 3) identified three groups of assemblages: Group 1 (AMX2/Cu2
373 and AMX2/Cu1), Group 2 (UC, AMX1/Cu2, Cu1, AMX1, and AMX1), and Group 3 (UC
374 together with the other treatments).

375 SIMPER analysis (Table 5) revealed a reduction in the relative abundance of conical
376 tails in all contaminated treatments except AMX1/Cu2. Conversely, all treatments showed an
377 increase in the proportion of clavate tails, along with a decline in the occurrence of nematodes
378 with elongated/filiform tails (see Table 5). The treatments AMX2/Cu2 (26.37%) and
379 AMX2/Cu1 (24.60%) showed the highest average dissimilarity compared to the controls,
380 indicating an overall increase in dissimilarity with rising contamination levels of both copper
381 and amoxicillin (refer to Table 5).

382 The second-stage nMDS (see Fig. 4) indicated that trophic groups were significantly
383 influenced by contamination (90.039%), while tail shape was comparatively less impacted
384 (83.126%).

385

386 *3.5. Computational findings*

387 The binding affinities of amoxicillin for GLD-3 and SDP are presented in Table 6,
388 showing negative docking of -6.4 and -7.7 kcal/mol, respectively. It was initially anticipated
389 that amoxicillin would form at least four conventional hydrogen bonds; however, it formed six
390 typical hydrogen bonds with SDP. Specifically, it interacted three times with LYS82, and once
391 each with GLN175, ASP166, and GLN78. The hydrogen bonds formed with GLD-3 were
392 accompanied by a π-Alkyl interaction with ARG113 (refer to Figs. 5 and 6). Our research
393 indicates that amoxicillin is precisely positioned (<2.5 Å) within the binding sites of both GLD-

3 and SDP, with interaction distances of 1.994 and 1.875 Å, respectively, to the nearest interacting residues of the receptors.

4. Discussion

The objective of this study was to evaluate the impact of amoxicillin [550 ng/L (AMX1) and 1100 ng/L (AMX2)] or copper [130 mg/kg dry weight (dw) (Cu1) and 260 mg/kg dw (Cu2)], and their combined mixtures (AMX1/Cu1, AMX2/Cu2, AMX1/Cu2, and AMX2/Cu1) on the abundance of meiofauna, and taxonomic and functional diversity of nematodes collected from the Jeddah coast, Saudi Arabia.

4.1. How did the presence of meiobenthic organisms alter under stress exposure?

A general decline in meiobenthic organisms was observed following exposure to copper and/or amoxicillin, with the most significant impact on nematodes occurring under the highest concentrations of amoxicillin, particularly in AMX2/Cu2, AMX2, and AMX2/Cu1.

Overall, copper is commonly associated with reduced population densities and elevated mortality rates in aquatic species like fungi (*Clavariopsis aquatica*), zooplankton (*Mytilinia* sp.), and macroinvertebrates (*Gammarus pulex*), potentially inducing behavioral changes (Environment Canada, 1998; Sandrine et al., 2009). In general, meiobenthic organisms are affected by stressors either through direct exposure to contaminated sediments or *via* ingestion of polluted particles (Armynot du Châtelet et al., 2016; Lassoued et al., 2025). It has been demonstrated that both dissolved and particulate forms of copper in seawater can be readily absorbed by sediments, whether bound to drugs and organic matter or not (Hung et al., 2024).

Alternatively, several indirect mechanisms may help explain the observed decline in meiobenthic taxa. For example :

(1) The accumulation of dead bodies from sensitive benthic species can reduce sediment porosity and increase adhesion (Allouche et al., 2025), thereby hindering the movement of meiobenthos for reproduction and feeding (Hedfi et al., 2008; Allouche et al., 2020), particularly impacting crustaceans and polychaetes that have a flattened body, presenting a large surface area exposed to xenobiotics.

(2) Bashir et al. (2020) reported that the disposal of copper mine tailings in the ocean led to the degradation of the coastal marine environments by decreasing light penetration and photosynthetic activity, resulting in increased mortality among algae, invertebrates, and fish.

(3) Finally, chronic exposure to copper in marine sediments have been shown to caused DNA damage and elevated mortality in the polychaete *Alitta virens* (Watson et al., 2018).

In general, the polychaetes, copepods, isopods, amphipods, and cumaceans appear to be the most vulnerable taxa, likely due to the high number of setae on their bodies, which impairs movement in such sticky, contaminated substrates (Sbrocca et al., 2021; Schratzberger et al., 2023).

At this stage, it becomes essential to investigate the effects of amoxicillin, copper, and their combinations on the taxonomic diversity of free-living marine nematodes, in order to distinguish between sensitive indicator species and tolerant ones.

4.2. How do nematodes react to stress from a taxonomic standpoint?

Significant declines in diversity indices and/or species richness were observed particularly in treatments AMX2/Cu2, AMX2, AMX2/Cu1, and AMX1/Cu2 (see Fig. 1). This reduction is undoubtedly attributable to the detrimental effects of these pollutants on sensitive species. Hedfi et al. (2008) reported a marked reduction in the taxonomic diversity of meiobenthic nematodes along the Bizerte shores (Tunisia), associated with increasing copper levels in marine sediments. More broadly, Environment Canada (1998) and Lee and Correa (2007) also recorded diversity loss in benthic invertebrates and meiofauna following exposure to copper or mine tailings. Regard amoxicillin, it has been shown to negatively affect aquatic organisms within the food web, particularly cyanobacteria, which are more sensitive than green algae (Andreozzi et al., 2004; González-Pleiter et al., 2013). Moreover, amoxicillin may stimulate *M. aeruginosa* proliferation and microcystin production, potentially leading to harmful algal blooms (Liu et al., 2016).

After exposure to copper, the species *P. sadspitensis*, *D. timmi*, *C. papillata*, *E. obtusicaudatus*, *T. longicaudata*, *T. poloris*, *H. longicaudatus*, *P. beviseta*, and *T. pertenuis* could be considered as sentinel species, due to distinct sensitivity. The epistrate feeder *Paracanthonchus sadspitensis* and *D. timmi* were particularly affected, likely due to the decline in diatoms, their main food source. This finding is consistent with Sandrine et al. (2009), who reported increased mortality in the diatom *Cocconeis* sp. at copper concentrations above 25 $\mu g.L^{-1}$. In contrast, *D. oxycerca* increased in abundance under high copper concentrations compared to the controls, suggesting a tolerance or adaptation to metal-enriched conditions. This may be linked to its ability to consume macrophyte remains (Grassi et al., 2023; Cocozza di Montanara et al., 2024). Sandrine et al. (2009) also suggested that copper contamination may enhance the growth of certain macrophytes, such as *Callitriche platycarpa*. Moreover, non-selective deposit feeders may benefit from feeding on decaying organic matter derived from sensitive taxa (Allouche et al., 2025).

Similarly, exposure to amoxicillin negatively affected six species: *C. papillata*, *T. longicaudata*, *H. longicaudatus*, *T. pertenuis*, *D. timmi*, and *V. viscosia*. This is likely linked to amoxicillin's mechanism of action, which reduces bacterial availability for the microvorous species such as *C. papillata*, *T. longicaudata*, and *H. longicaudatus*. Nasri et al. (2015) observed significant shifts in both taxonomic and trophic composition in a Mediterranean benthic community exposed to penicillin G under laboratory conditions. In contrast, some species, such as *D. oxycerca* and *M. albidus,* appeared as opportunistic/tolerant taxa for amoxicillin. This is consistent with their trophic roles as obligate or facultative detritivores.

The most pronounced impacts on nematode taxonomic composition were observed in the combined and high-concentration treatments, particularly AMX2/Cu2 (see Fig. 1 and Table 4). This was futher supported by the nMDS ordination, which showed a clear separation between single and combined treatments. Under mixed exposures to copper and amoxicillin, several species, including *E. obtusicaudatus, T. pertenuis, T. longicaudata, H. longicaudatus, and Trissonchulus oceanus*, disappeared. The latter species may shift toward detritivory when organic matter from dead organisms is present (Moens et al., 2006). This finding is supported by reductions in the populations of other species such as *C. papillata*, *T. poloris*, *Daptonema conicum*, *Paramonhystera pellucida*, *P. beviseta*, and *Sabatieria falcifera*. These results suggest a potential synergistic or additive effect of amoxicillin and copper on taxonomic composition of meiobenthic nematodes. The dissimilarity values supports this hypothesis (refer to Table 4), as they were notably higher in the high-concentration mixture compared to single treatments or the control. Several studies support the idea of synergistic interactions between copper and amoxicillin. For instance, copper has been shown to enhance the antibacterial activity of amoxicillin (Khashan et al., 2015; Nleonu et al., 2022; Su et al., 2024). Su et al. (2024) demonstrated that the co-application of amoxicillin and copper during composting reduced humification, with stronger negative effects when used together than individually. Copper-

amoxicillin complexes were found to be more effective agaist *Staphylococcus aureus* than amoxicillin alone (Nleonu et al., 2022). Likewise, copper oxide nanoparticles showed strong antibacterial effects against *E. coli* and *S. aureus*, with enhanced efficacy when combined with amoxicillin (Khashan et al., 2015).

4.3. How do nematodes react to stress from a functional standpoint?

Examining functional traits offers essential insight into ecotoxicological responses, although this aspect is often overlooked by meiobenthic nematologists (Semprucci et al., 2022; Allouche et al., 2025). At the conclusion of the present bioassay, the greatest dissimilarities in feeding were observed between contaminated treatments and controls, particularly in combined treatments at higher concentrations (AMX2/Cu2 and AMX2/Cu1). Similarly, the most significant changes in tail morphotypes were also recorded under these same conditions.

In these treatments, predators (2B) were found in higher proportions, possibly indicating that the effects of amoxicillin and copper became more pronounced toward the later phase of the experimental. These worms are known to feed fresh dead prey (Moens et al., 2002; Allouche et al., 2021 a,b), suggesting that mortality among other taxa may have provided an abundant food source. Additionally, nematodes with clavate-shaped tails became more prevalent, whereas those with conical or elongated/filiform tails generally declined or disappeared. This is consistent with the idea that conical-tailed nematodes, which rely on a broader base to leap and move, are more exposed to contaminants adsorbed onto sediment particles (Bellakhal et al., 2023). Meanwhile, nematodes with elongated tails face difficulties in foraging under sticky and compacted sediment conditions, which are characteristic of contaminated environments.

The second-stage nMDS ordination (refer to Fig. 4) confirmed that the distribution of nematode species following exposure to amoxicillin and/or copper was primarily associated

514 with their buccal cavity, and to a lesser degree with tail shape. Notably, microvorous nematodes
515 were among the most affected groups. Amoxicillin, in fact, has been shown to significantly
516 impact aquatic ecosystems, with cyanobacteria being especially vulnerable, as evidenced in
517 conventional ecotoxicological bioassays (González-Pleiter et al., 2013; Kovaláková et al.,
518 2020). For instance, amoxicillin is harmful to the *Cyanobacterium anabaena* CPB4337, while
519 it does not affect the green alga *Pseudokirchneriella subcapitata* (González-Pleiter et al., 2013),
520 and may even stimulate its photosynthetic activity (Liu et al., 2016). This pattern helps explain
521 the relatively high abundance of obligate (1B) and facultative (2B) herbivorous nematodes,
522 which may have benefited from altered microbial and primary producer communities resulting
523 from amoxicillin exposure.

524

525 *4.4. Is there support from computational modeling for both taxonomic and functional traits?*

526 Table 6 presents the binding affinities of amoxicillin to GLD-3 and SDP. The results
527 indicated negative scores, with satisfactory binding affinities of –6.4 for GLD-3 and –7.7
528 kcal/mol for SDP. Negative binding energies, particularly those below –6.0 kcal/mol, are
529 frequently associated with biologically relevant effects (Allouche et al., 2022; Ishak et al.,
530 2024). Given that hydrogen bonds play a fundamental role in mediating biological activity
531 (Badraoui et al., 2024; Elyousfi et al., 2024), it was hypothesized that amoxicillin would
532 establish at least four conventional hydrogen bonds. In practice, it formed six hydrogen bonds
533 when bound to SDP, interacting three times with LYS82 and once with each of GLN175,
534 ASP166, and GLN78. In case of GLD-3, the established hydrogen bonds were accompained by
535 a π-alkyl interaction with ARG113 (Figures 5 and 6). Hydrogen bonding and other non-covalent
536 interactions are recognized as critical determinants of the toxicological effects of several
537 compounds, including antibiotics (Ishak et al., 2024; Ben Amor et al., 2025). Our results showed

that amoxicillin was tightly embedded within the active sites of both GLD-3 and SDP, with interaction distances of 1.994 Å and 1.875 Å, respectively, to the nearest binding residues. Such close molecular embedding is known to enhance the biological activity and stability of ligand–receptor complexes (Ben Amor et al., 2025; Rahmouni et al., 2024; Elyousfi et al., 2024). The strong molecular interaction of amoxicillin with these two nematode receptors provides a mechanistic explanation that supports the experimentally observed reduction in taxonomic diversity and species prevalence, especially under high concentrations and in combination with copper.

5. Conclusions

This study evaluated the effects of amoxicillin, copper, and their mixtures on meiobenthic organisms, with a particular focus on nematodes. A notable decrease in meiobenthic abundance was observed, alongside a significant decline in taxonomic diversity, especially under elevated concentrations and combined exposures. Some species emerged as indicators of pollution, including *D. oxycerca* (for copper) and *M. albidus* (for both). In constrast, several taxa were recognized as sensitive species: *P. sadspitensis*, *D. timmi*, *C. papillata*, *E. obtusicaudatus*, *T. longicaudata*, *T. poloris*, *H. longicaudatus*, *P. beviseta*, and *T. pertenuis* for copper, and *C. papillata*, *T. longicaudata*, *H. longicaudatus*, *T. pertenuis*, *D. timmi*, and *V. viscosia* for amoxicillin. The results further confirmed the higher sensitivity of polychaetes and crustaceans compared to nematodes to both contaminants, and highlighted the presence of synergistic/additive effects between these two chemicals. These interactions may be explained by electrostatic affinities between the two substances, potentially enhancing their toxicological impact.

In summary, the research underscores the negative cosenquences of pharmaceutical and metal contamination on meiobenthic biodiversity, emphasizing the need for further targeted

studies. Future research should employ mono-species bioassays, focusing on sensitive nematode taxa exposed to amoxicillin and/or copper, and should include detailed assessments of mortality, morphotyping, and genetic haplotyping.

Acknowledgments

This work was supported and funded by the Deanship of Scientific Research at Imam Mohammad Ibn Saud Islamic University (IMSIU) (grant number IMSIU-DDRSP2502).

Conflict of interest: On behalf of all authors, the corresponding author states that there is no conflict of interest.

Ethical approval: Not applicable**.**

Consent for publications: The Author grants the Publisher the sole and exclusive license of full copyright.

Consent to participate: All authors read and approved the final version of the manuscript being submitted.

Data availability: The data supporting this study's findings are available from the corresponding author upon reasonable request.

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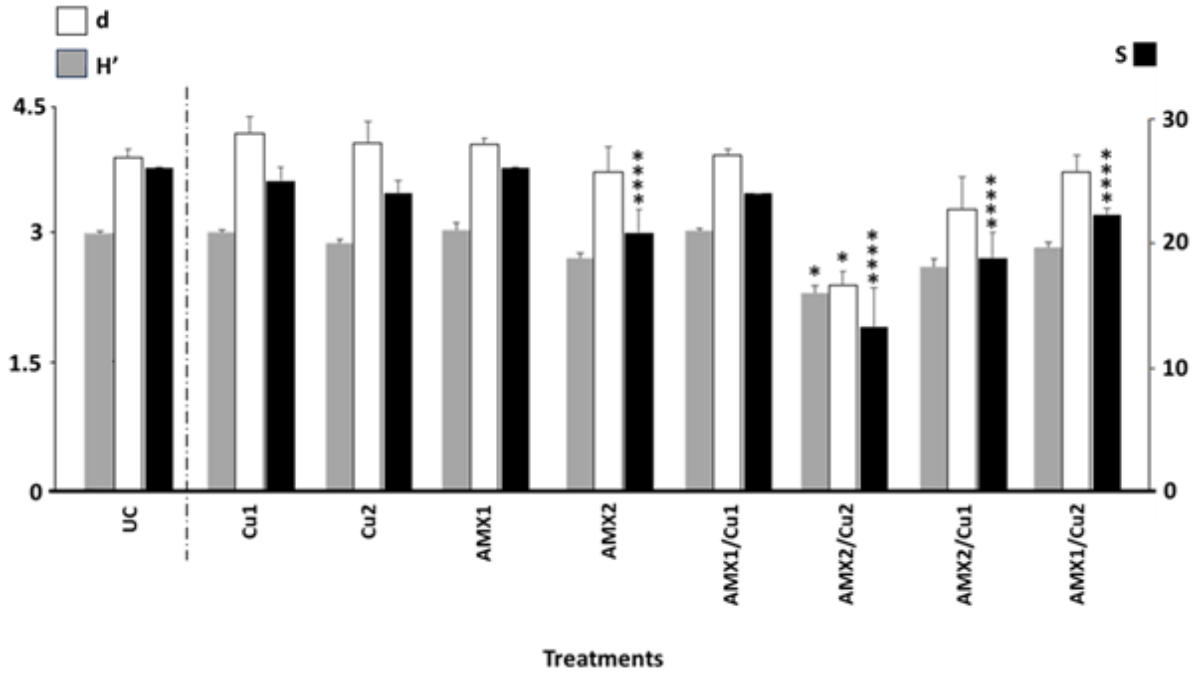
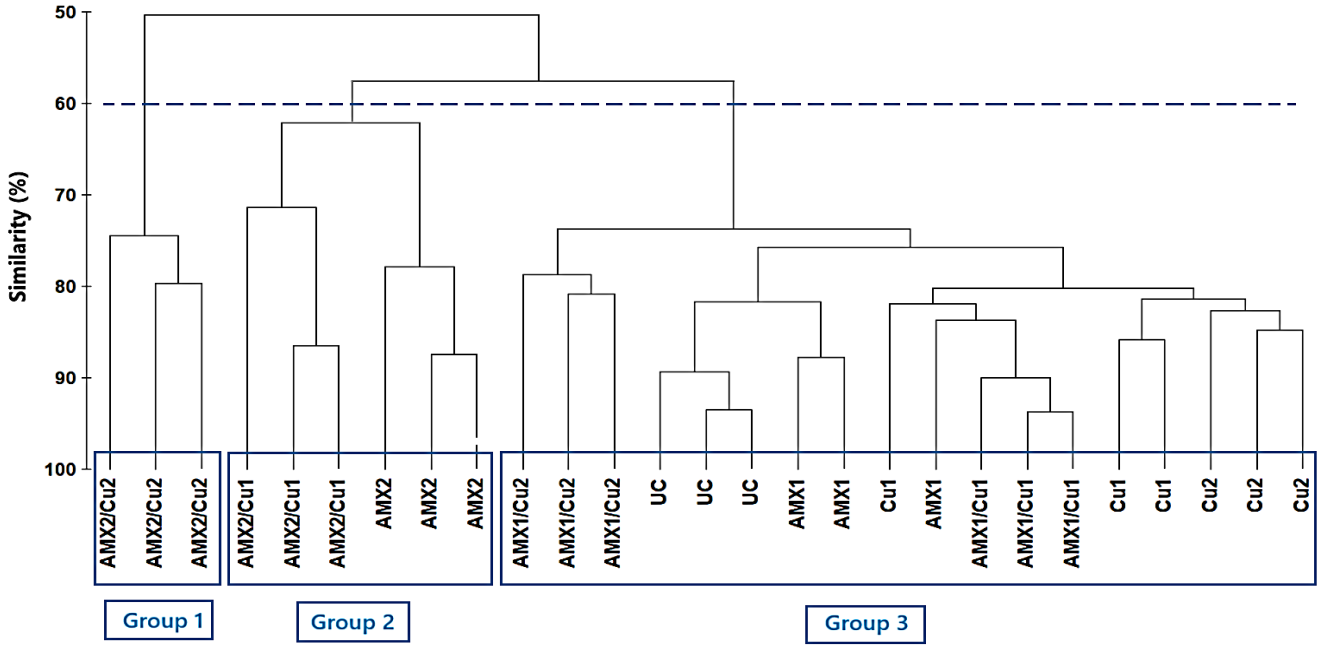


Fig. 1. Graphical representation of univariate indices for nematode communities from the control treatment (UC) and in treatments enriched with amoxicillin [550 ng/L (AMX1) and 1100 ng/L (AMX2)], copper [130 mg/kg Dry Weight 'dw' (Cu1) and 260 mg/kg dw (Cu2)] and their mixtures (AMX1Cu1, AMX2Cu2, AMX2Cu1, and AMX1Cu2). Taxonomic indices: H' = Shannon-Wiener index, d = Margalef's, species richness, S = species number. Tukey's HSD test : $p < 0.05$ (*), $p < 0.0001$ (****).



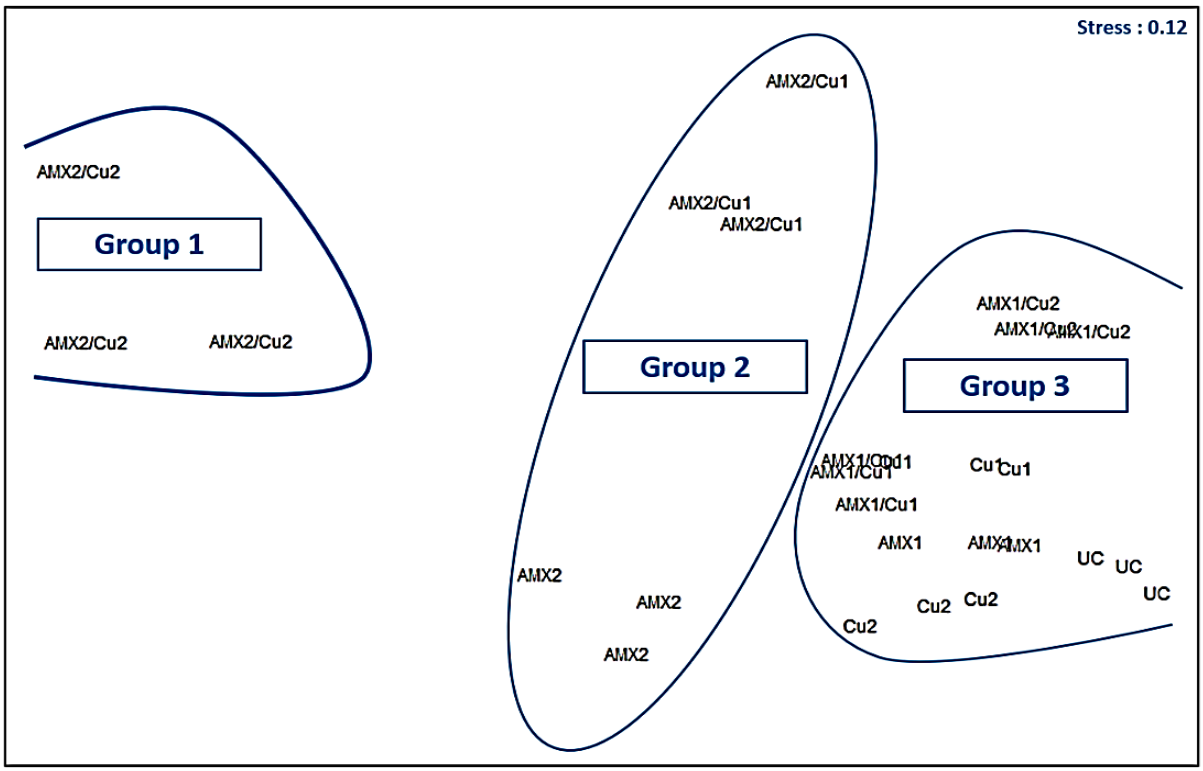


Fig. 2. Two-dimensional non-metric multidimensional scaling (nMDS) plot based on nematode species composition from the control treatment (UC) and treatment enriched with amoxicillin [550 ng/L (AMX1) and 1100

ng/L (AMX2)], copper [130 mg/kg Dry Weight 'dw' (Cu1) and 260 mg/kg dw (Cu2)] and their mixtures (AMX1Cu1, AMX2Cu2, AMX2Cu1, and AMX1Cu2).

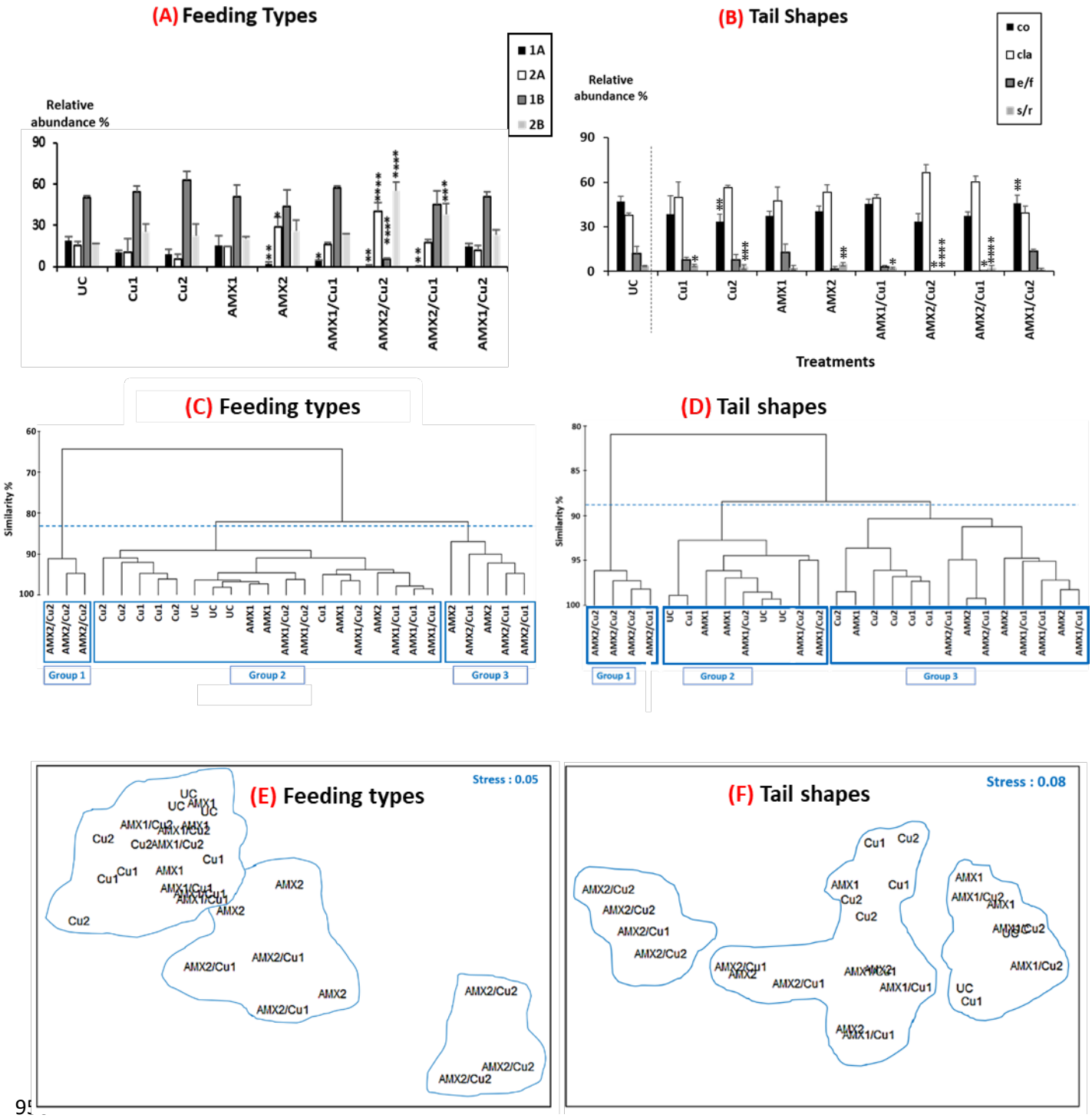


Fig. 3. Changes in the functional traits of nematodes (trophic groups and tail shapes) in the control treatment (UC) and treatments enriched with amoxicillin [550 ng/L (AMX1) and 1100 ng/L (AMX2)], copper [130 mg/kg Dry Weight 'dw' (Cu1) and 260 mg/kg dw (Cu2)] and their mixtures (AMX1Cu1, AMX2Cu2, AMX2Cu1, and AMX1Cu2) over a 30 days period. Visual summary based on relative abundances (A and B), cluster analyses (C and D), and non-metric multidimensional scaling (nMDS) representations (E and F). Feeding groups: selective deposit feeders (1A), non-selective deposit feeders (1B), epigrowth feeders (2A), omnivorous-carnivorous (2B).

963 Tail shapes: elongated/filiform (e/f), short/round (s/r), conical (co), clavate/cylindrical (cla). Asterisks above bars
964 indicate significant differences compared to the control groups, according to the Chi-square test results (√-
965 transformed data) : $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), $p < 0.0001$ (****).

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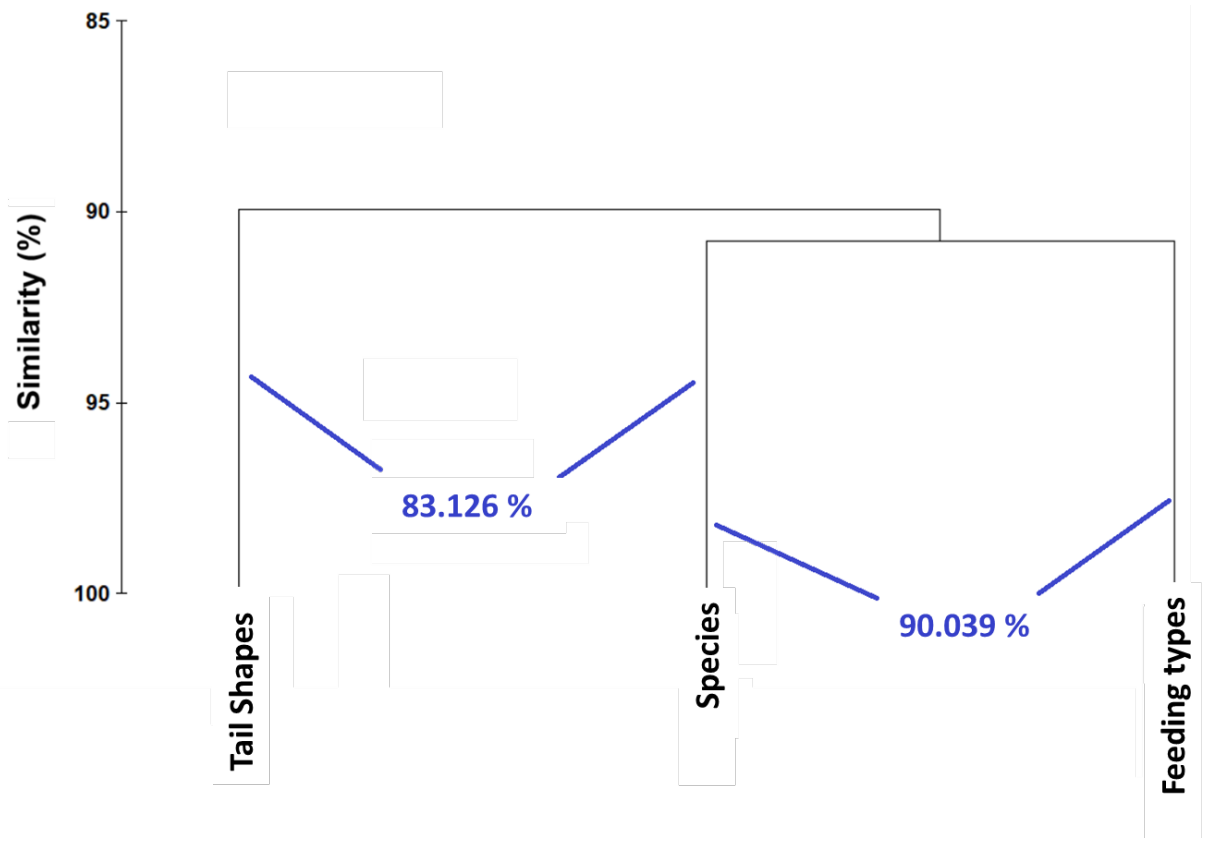

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969 **Fig. 4.** Second-stage clustering based Spearman rank correlations derived from inter-matrix
970 comparisons. Included matrices are those represented in Figures 2 (species composition) and 3
971 (functional traits). Average similarity values are indicated on the dendrogram branches.

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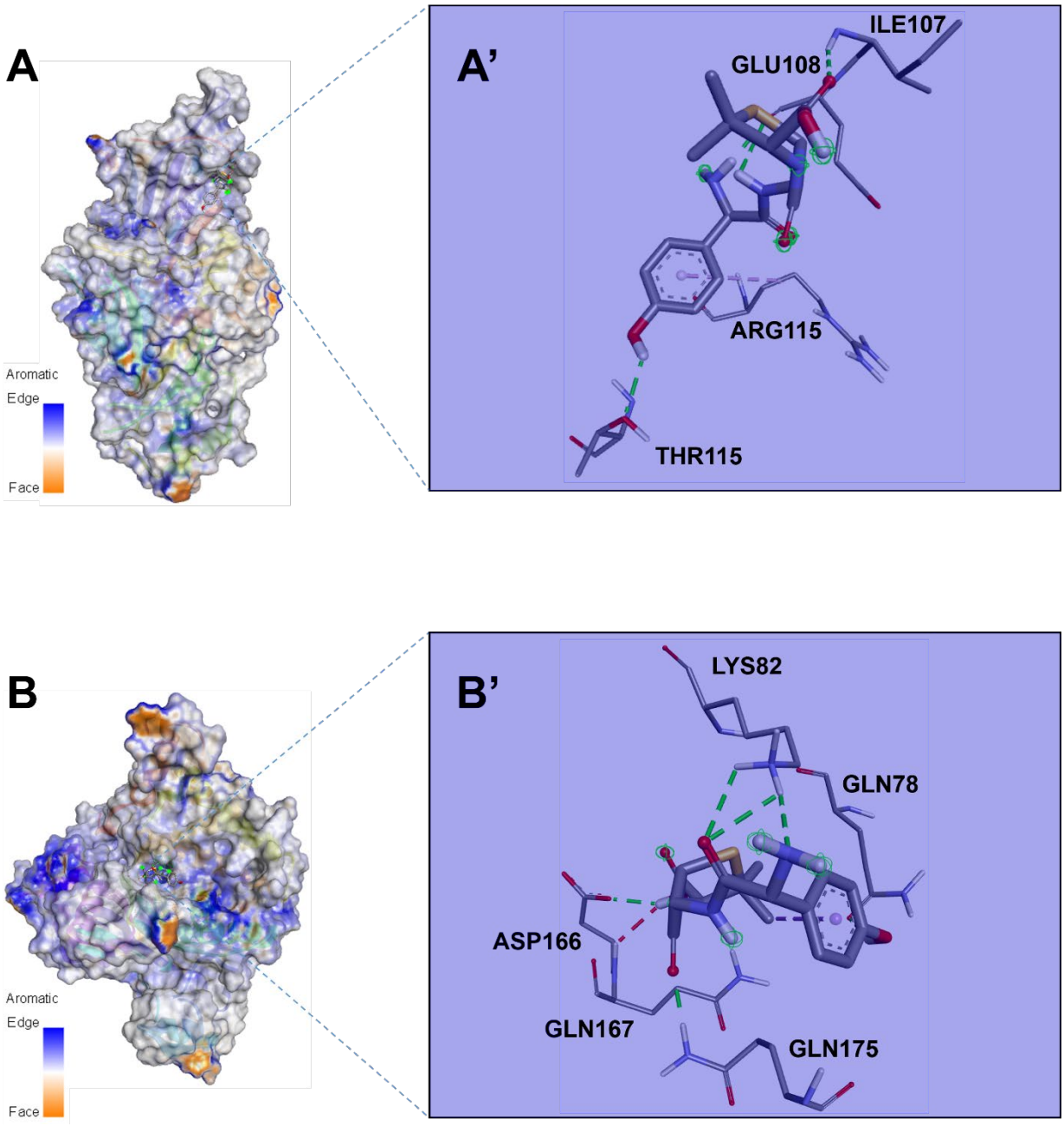
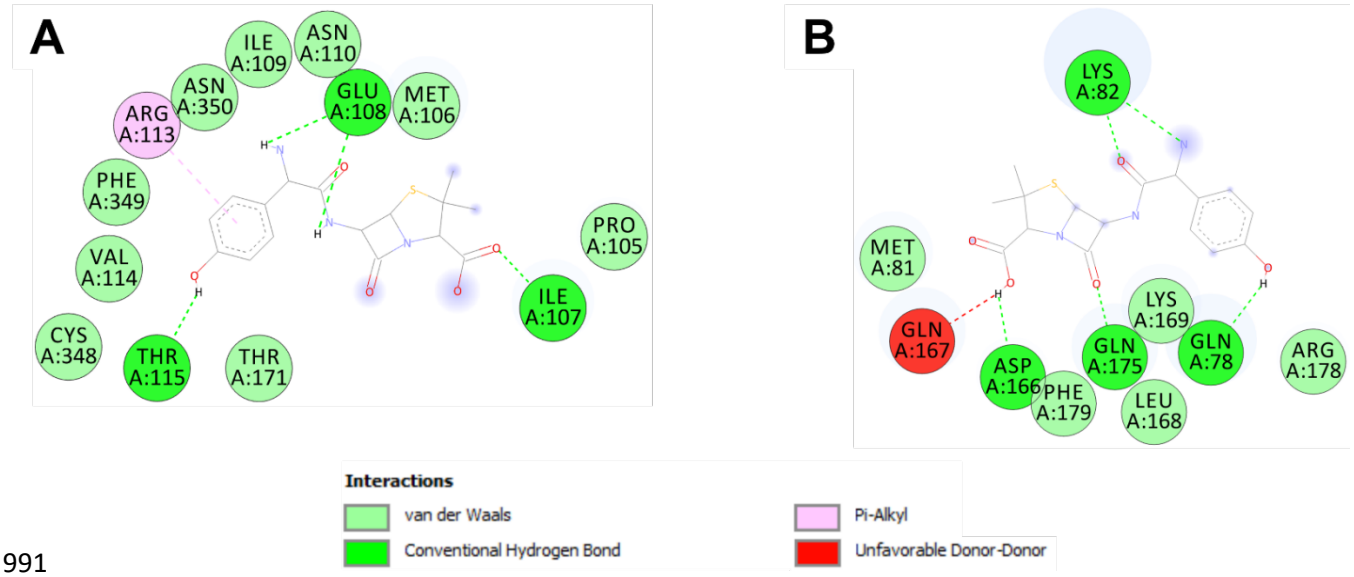


Fig. 5. 3D illustrations of amoxicillin complexed with (**A**) Germline Development Protein 3 (GLD-3) and (**B**) Sex-Determining Protein (SDP) of *Caenorhabditis elegans*, used as a nematode model. Panels (A') and (B') show the corresponding zoomed-in views of the molecular interactions within each complex.



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993 **Fig. 6.** Bidimensional interactions diagrams amoxicillin complexed with (**A**) Germline Development Protein 3
994 (GLD-3) and (**B**) Sex-Determining Protein (SDP) of *Caenorhabditis elegans*, used as a nematode model.

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Table 1. Abundances of meiobenthic taxa in the control treatment (UC) and those enriched with amoxicillin [550 ng/L (AMX1) and 1100 ng/L (AMX2)] or copper [130 mg/kg Dry Weight 'dw' (Cu1) and 260 mg/kg dw (Cu2)] and their mixtures (AMX1Cu1, AMX2Cu2, AMX2Cu1, and AMX1Cu2). Significant differences compared to the control are indicated using Tukey's test: $p < 0.05$ (*), $p < 0.01$ (**), $p < 0.001$ (***), and $p < 0.0001$ (****). Log(x)-transformed data are shown in black zones, and log(x+1)-transformed data in gray zones.

Meiobenthic groups	UC	Cu1	Cu2	AMX1	AMX2	AMX1/Cu1	AMX2/Cu2	AMX2/Cu1	AMX1/Cu2
Nematodes	504 ± 84	284 ± 30 (**)	232 ± 20 (**)	393 ± 43 (*)	169 ± 11 (***)	256 ± 23 (**)	132 ± 6 (***)	180 ± 15 (***)	246 ± 33 (**)
Polychaetes	73 ± 7	24 ± 4 (*)	15 ± 6 (**)	11 ± 8 (**)	7 ± 1 (****)	13 ± 7 (**)	0 (****)	0 (****)	6 ± 4 (****)
Copepods	101 ± 14	66 ± 10 (**)	47 ± 17 (**)	58 ± 11(**)	12 ± 8 (****)	9 ± 0 (****)	0 (****)	0 (****)	0 (****)
Isopods	75 ± 19	60 ± 19	38 ± 14 (**)	0 (****)	0 (****)	7 ± 5 (****)	5 ± 4 (****)	0 (****)	3 ± 1 (****)
Cumacea	12 ± 6	5 ± 2 (*)	0 (****)	7 ± 1 (***)	0 (****)	8 ± 4	0 (****)	0 (****)	0 (****)
Amphipods	106 ± 27	85 ± 13 (*)	29 ± 8 (***)	0 (****)	0 (****)	0 (****)	0 (****)	0 (****)	0 (****)

Table 2. List of nematode species and their associated functional traits collected from a pristine site along the coast of Jeddah in Saudi Arabia, on March 17, 2023, accross the different microcosms. Tail morphotypes: elongated/filiform (e/f), conical (co), clavate/conical-cylindrical (cla), short/rounded (s/r). Feeding types: selective deposit feeders (1A), non-selective deposit feeders (1B), epigrowth feeders (2A), omnivores-carnivores (2B).

Species	Order	Family	Tail shape	Trophic group
Cinctonema papillata	Desmodorida	Microlaimidae	co	1A
Daptonema conicum	Monhysterida	Xyalidae	cla	1B
Daptonema oxycerca	Monhysterida	Xyalidae	cla	1B
Desmodora pontica	Desmodorida	Desmodoridae	co	2A
Dorylaimopsis timmi	Araeolaimida	Comesomatidae	cla	2A
Eleutherolaimus obtusicaudatus	Monhysterida	Linhomoeidae	co	1B
Halalaimus longicaudatus	Enoplida	Oxystominidae	e/f	1A
Halichoanolaimus balochiensis	Chromadorida	Selachinematidae	cla	2B
Metoncholaimus albidus	Enoplida	Oncholaimidae	cla	2B
Metoncholaimus pristiurus	Enoplida	Oncholaimidae	cla	2B
Oncholaimus campylocercoides	Enoplida	Oncholaimidae	cla	2B
Paracanthonchus sadspitensis	Chromadorida	Cyatholaimidae	co	2A
Paramonhystera pellucida	Monhysterida	Xyalidae	cla	1B
Parodontophora breviseta	Araeolaimida	Axonolaimidae	co	1B
Parodontophora pacifica	Araeolaimida	Axonolaimidae	co	1B
Ptycholaimellus sindhicus	Chromadorida	Chromadoridae	co	2A
Sabatieria falcifera	Araeolaimida	Comesomatidae	cla	1B
Terschellingia communis	Monhysterida	Xyalidae	e/f	1A
Terschellingia longicaudata	Monhysterida	Xyalidae	e/f	1A
Theristus flevensis	Monhysterida	Xyalidae	co	1B
Theristus pertenuis	Monhysterida	Xyalidae	co	1B
Theristus poloris	Monhysterida	Xyalidae	co	1B
Trissonchulus oceanus	Enoplida	Ironidae	s/r	1B
Viscosia macramphida	Enoplida	Oncholaimidae	cla	2B
Viscosia viscosa	Enoplida	Oncholaimidae	cla	2B

Table 3. Relative abundance (±SD) of free marine nematodes species from in the control treatment (UC) and in treatments enriched with amoxicillin [550 ng/L (AMX1) and 1100 ng/L (AMX2)] or copper [130 mg/kg Dry Weight 'dw' (Cu1) and 260 mg/kg dw (Cu2)] and their mixtures (AMX1Cu1, AMX2Cu2, AMX2Cu1, and AMX1Cu2) after 30 days of the exposure.

Species	UC	Cu1	Cu2	AMX1	AMX2	AMX1/Cu1	AMX2/Cu2	AMX2/Cu1	AMX1/Cu2
Cinctonema papillata	7.27 ± 2.98	2.27 ± 1.05	1.42 ± 0.43	2.45 ± 2.19		1.32 ± 0.50	0.52 ± 0.91		1.17 ± 0.60
Daptonema conicum	5.50 ± 0.11	6.51 ± 2.36	2.44 ± 1.34	9.75 ± 3.34	1.76 ± 0.99	2.38 ± 0.58	0.48 ± 0.83	10.22 ± 10.32	3.45 ± 2.74
Daptonema oxycerca	0.88 ± 0.50	7,30 ± 2.30	10.27 ± 1.63	5.20 ± 2.60	12.42 ± 0.84	9.48 ± 1.45			1.62 ± 1.69
Desmodora pontica	2.14 ± 0.94	4,59 ± 2.15	3.55 ± 2.12	3.87 ± 0.93	8.99 ± 2.27	3.73 ± 0.35	13.60 ± 2.85	11.91 ± 4.30	7.39 ± 2.56
Dorylaimopsis timmi	5.75 ± 1.88	0.90 ± 0.83	0.93 ± 1.62	1.70 ± 1.35	3.54 ± 1.53	4.64 ± 2.10	10.35 ± 7.33	1.95 ± 1.88	
Eleutherolaimus obtusicaudatus	8.59 ± 0.78	5.98 ± 4.30	9.52 ± 2.40	8.14 ± 1.22	7.69 ± 7.34	8.10 ± 0.63		5.01 ± 3.94	8.74 ± 0.91
Halalaimus longicaudatus	5.33 ± 2.28	2.96 ± 0.79	2.17 ± 0.79	4.26 ± 3.35	0.55 ± 0.96				1.50 ± 1.71
Halichoanolaimus balochiensis	3.79 ± 0.45	4.67 ± 1.90	3.11 ± 1.10	4.47 ± 1.59	3.91 ± 3.68	3.25 ± 0.57	6.87 ± 7.65	4.20 ± 3.79	4.22 ± 4.01
Metoncholaimus albidus	1.70 ± 0.55	3.65 ± 2.56	5.07 ± 1.33	5.30 ± 0.82	8.00 ± 2.80	4.77 ± 0.87	13.65 ± 3.41		
Metoncholaimus pristiurus	1.48 ± 0.65	5.90 ± 1.45	1.44 ± 0.51	2.56 ± 0.98	2.98 ± 1.27	4.57 ± 1.62	6.62 ± 5.04	13.95 ± 2.44	8.10 ± 2.05
Oncholaimus campylocercoides	2.23 ± 0.72	1.51 ± 0.86	4.52 ± 3.73	2.19 ± 0.83	3.80 ± 3.05	2.86 ± 0.82	14.87 ± 2.13	8.46 ± 3.16	0.38 ± 0.37
Paracanthonchus sadspitensis	6.34 ± 0.65	0.82 ± 1.43	0.90 ± 0.88	5.73 ± 0.78	13.16 ± 3.33	2.32 ± 0.48	9.14 ± 1.69	2.05 ± 0.43	1.48 ± 0.73
Paramonhystera pellucida	4.64 ± 1.11	4.45 ± 1.14	7.05 ± 1.50	5.19 ± 2.68	4.39 ± 1.58	5.09 ± 1.09	0,48 ± 0.83	4.58 ± 4.12	5.26 ± 2.23
Parodontophora beviseta	4.46 ± 1.54	3.22 ± 0.58	3.55 ± 0.76	2.77 ± 0.69	3.37 ± 0.96	3.61 ± 0.54	0.24 ± 0.42	2.73 ± 0.96	4.65 ± 1.60
Parodontophora pacifica	4.18 ± 0.47	3.37 ± 2.32	4.33 ± 2.20	4.10 ± 1.20	1.94 ± 0.77	5.11 ± 1.48	1.81 ± 2.55	2.59 ± 1.62	5.20 ± 2.50
Ptycholaimellus sindhicus	0.67 ± 0.47	4.06 ± 5.40		3.03 ± 1.96	3.36 ± 0.28	5.47 ± 2.47	6.63 ± 1.85	1.44 ± 1.09	2.59 ± 0.94
Sabatieria falcifera	4.61 ± 0.49	5.16 ± 3.22	12.97 ± 4.45	5.83 ± 5.80	5.35 ± 3.44	4.99 ± 2.24	0.48 ± 0.83	5.86 ± 3.27	5.70 ± 2.47
Terschellingia communis	0.83 ± 0.31	2.31 ± 1.31	3.88 ± 3.12	5.67 ± 2.83	0.39 ± 0.64	2.88 ± 1.20			1.78 ± 1.94
Terschellingia longicaudata	5.69 ± 2.74	2.49 ± 1.08	1.60 ± 0.73	2.65 ± 0.62	0.60 ± 0.62			0.17 ± 0.30	10.18 ± 4.76
Theristus flevensis	1.25 ± 0.09	5.21 ± 2.41	2.95 ± 1.36	2.90 ± 1.07	0.80 ± 0.38	3.94 ± 0.86	1.29 ± 2.24	5.95 ± 4.35	5.10 ± 4.66
Theristus pertenuis	6.29 ± 0.24	5.84 ± 0.44	4.50 ± 1.96	1.80 ± 1.63	1.16 ± 1.01	4.95 ± 0.63		2.14 ± 1.87	5.15 ± 1.63
Theristus poloris	5.80 ± 0.72	3.1 ± 0.56	2.68 ± 1.56	2.66 ± 1.36		7.10 ± 1.70	0.24 ± 0.42	3.62 ± 1.75	4.58 ± 1.90
Trissonchulus oceanus	3.48 ± 0.39	4.00 ± 0.66	2.70 ± 1.61	2.37 ± 1.50	4.78 ± 1.25	2.19 ± 0.66		2.10 ± 1.89	1.34 ± 0.85
Viscosia macramphida	2.48 ± 0.42	5,65 ± 3.90	3.78 ± 2.38	3.22 ± 0.09	6.66 ± 3.56	3.38 ± 0.38	4.54 ± 0.56	4.12 ± 1.77	3.53 ± 1.39
Viscosia viscosa	4.58 ± 0.17	4.08 ± 2.47	4.68 ± 3.54	2.20 ± 0.19	0.41 ± 0.72	3.88 ± 0.54	8.18 ± 2.66	6.94 ± 2.01	6.90 ± 2.71

Table 4. Analysis of similarity (ANOSIM) and percent similarity (SIMPER) comparing the control treatment (UC) with treatments enriched with amoxicillin [550 ng/L (AMX1) and 1100 ng/L (AMX2)], copper [130 mg/kg Dry Weight 'dw' (Cu1) and 260 mg/kg dw (Cu2)] and their mixtures (AMX1Cu1, AMX2Cu2, AMX2Cu1, and AMX1Cu2). The table provides average dissimilarity (AD) values between treatments based on square root transformed nematode species abundance. Species contributing to aproximatively 70% of the total dissimilarity are listed in order of their contribution. Symbols: more present (+); less present (-); eliminated (elim).

Comparisons	UC *vs.* Cu1	UC *vs.* Cu2	UC *vs.* AMX1	Uc *vs.* AMX2
ANOSIM	*R*-statistics = 0.926 *p* = 0.01	*R*-statistics = 1 *p* = 0.01	*R*-statistics = 0.889 *p* = 0.01	*R*-statistics = 1 *p* = 0.01
SIMPER	AD = 25.92%	AD = 28.70%	AD = 19.37%	AD = 39.33%
	Paracanthonchus sadspitensis (10.27%) - *Dorylaimopsis timmi* (8.76%) - *Cinctonema papillata* (7.75%) - *Eleutherolaimus obtusicaudatus* (6.28%) - *Terschellingia longicaudata* (5.73%) - *Theristus poloris* (5.47) - *Halalaimus longicaudatus* (4.91%) - *Daptonema oxycerca* (4.82%) + *Parodontophora breviseta* (3.82%) - *Theristus pertenuis* (3.72%) – *Parodontophora pacifica* (3.69%) - *Viscosia viscosa* (3.57%) -	*Dorylaimopsis timmi* (9.03%) - *Paracanthonchus sadspitensis* (8.97%) - *Cinctonema papillata* (8.35%) - *Terschellingia longicaudata* (6.58%) - *Theristus poloris* (5.93%) - *Daptonema conicum* (5.84%) - *Daptonema oxycerca* (5.65%) + *Halalaimus longicaudatus* (5.64%) - *Theristus pertenuis* (4.88%) - *Eleutherolaimus obtusicaudatus* (3.77%) - *Parodontophora breviseta* (3.59%) -	*Theristus pertenuis* (8.31%) - *Cinctonema papillata* (7.92%) - *Dorylaimopsis timmi* (7.73%) - *Terschellingia communis* (6.70%) + *Daptonema oxycerca* (6.12%) + *Theristus poloris* (5.77%) - *Terschellingia longicaudata* (5.09%) - *Viscosia viscosa* (4.78%) - *Halalaimus longicaudatus* (4.41%) - *Sabatieria falcifera* (4.34%) - *Metoncholaimus albidus* (4.27%) + *Ptycholaimellus sindhicus* (4.20%) +	*Cinctonema papillata* (9.52%) *elim* *Theristus poloris*(8.56%) *elim* *Halalaimus longicaudatus* (7.13%) - *Theristus pertenuis* (7.11%) - *Terschellingia longicaudata* (6.96%) - *Viscosia viscosa* (6.85%) - *Daptonema conicum* (5.70%) - *Eleutherolaimus obtusicaudatus* (5.12%) - *Dorylaimopsis timmi* (4.68%) - *Parodontophora pacifica* (4.44%) -
Comparisons	UC *vs.* AMX1/ Cu1	UC *vs.* AMX2/Cu2	UC *vs.* AMX1/Cu2	UC *vs.* AMX2/Cu1
ANOSIM	*R*-statistics = 1 *p* = 0.01	*R*-statistics = 1 *p* = 0.01	*R*-statistics = 1 *p* = 0.01	*R*-statistics = 1 *p* = 0.01
SIMPER	AD = 24.87%	AD = 54.53%	AD = 29.18%	AD = 41.04%
	Terschellingia longicaudata (11.27%) elim *Halalaimus longicaudatus* (10.98%) elim *Cinctonema papillata* (8.89%) - *Daptonema oxycerca* (6.84%) + *Paracanthonchus sadspitensis* (6.71%) - *Daptonema conicum* (5.81%) - *Ptycholaimellus sindhicus* (4.60%) + *Theristus pertenuis* (4.08%) - *Dorylaimopsis timmi* (3.88%) - *Eleutherolaimus obtusicaudatus* (3.86%) - *Metoncholaimus pristiurus* (1.91%) +	*Eleutherolaimus obtusicaudatus* (8.35%) elim *Theristus pertenuis* (7.14%) elim *Cinctonema papillata* (7.00%) - *Terschellingia longicaudata* (6.60%) elim *Theristus poloris* (6.43%) - *Halalaimus longicaudatus* (6.43%) elim *Daptonema conicum* (6.09%) - *Paramonhystera pellucida* (5.53%) - *Parodontophora breviseta* (5.52%) - *Sabatieria falcifera* (5.52%) - *Trissonchulus oceanus* (5.30%) elim	*Dorylaimopsis timmi* (10.54%) elim *Cinctonema papillata* (8.52%) - *Paracanthonchus sadspitensis* (7.44%) - *Halalaimus longicaudatus* (7.03%) - *Metoncholaimus albidus* (5.69%) elim *Daptonema conicum* (5.05%) - *Oncholaimus campylocercoides* (4.99%) - *Trissonchulus oceanus* (4.74%) - *Theristus pertenuis* (4.12%) - *Theristus poloris* (4.06%) - *Theristus flevensis* (3.85%) + *Eleutherolaimus obtusicaudatus* (3.80%) -	*Cinctonema papillata* (9.18%) elim *Halalaimus longicaudatus* (7.75%) elim *Terschellingia longicaudata* (7.45%) - *Dorylaimopsis timmi* (5.83%) - *Theristus pertenuis* (5.80%) - *Paracanthonchus sadspitensis* (5.71%) - *Eleutherolaimus obtusicaudatus* (5.70%) - *Metoncholaimus albidus* (4.43%) elim *Theristus poloris* (4.39%) - *Trissonchulus oceanus* (4.00%) - *Paramonhystera pellucida* (3.85%) - *Parodontophora pacifica* (3.82%) -

Table 5. Analysis of similarity (ANOSIM) and percent similarity (SIMPER) comparing the control treatment (UC) with treatments enriched with amoxicillin [550 ng/L (AMX1) and 1100 ng/L (AMX2)], copper [130 mg/kg Dry Weight 'dw' (Cu1) and 260 mg/kg dw (Cu2)] and their mixtures (AMX1Cu1, AMX2Cu2, AMX2Cu1, and AMX1Cu2). The table reports average dissimilarity (AD) values between treatments based on square-root transformed abundances of trophic and tail shape groups. Functional groups contributing to approximately 70% of the total dissimilarity are listed in order of their contribution. Tail shapes: elongated/filiform (e/f), conical (co), clavate/conical-cylindrical (cla), short/rounded (s/r). Feeding types: selective deposit feeders (1A), non-selective deposit feeders (1B), omnivores–carnivores (2B). Symbols: more present (+); less present (–); eliminated (elim).

Comparisons		UC *vs.* Cu1	UC *vs.* Cu2	UC *vs.* AMX1	Uc *vs.* AMX2
Trophic groups	ANOSIM	AD = 15.91% *R*-statistics = 0.667 *p* = 0.01	AD = 19.90% *R*-statistics = 0.63 *p* = 0.01	AD = 8.79% *R*-statistics = 0.185 *p* = 0.01	AD = 26.11% *R*-statistics = 0.852 *p* = 0.01
	SIMPER	2B (28.86) + 1A (28.54) -	1B (33.32) + 1A (25.26) -	1B (34.65%) + 1A (31.84%) -	1A (33.71%) - 2B (27.06%) +
Tail shapes	ANOSIM	AD = 15.32% *R*-statistics = 0.667 *p* = 0.01	AD = 19.39% *R*-statistics = 0.63 *p* = 0.01	AD = 12.71% *R*-statistics = 0.185 *p* = 0.01	AD = 16.87% *R*-statistics = 0.852 *p* = 0.01
	SIMPER	cla (40.51%) + co (40.22%) - e/f (16.98%) -	cla (47.98%) + co (35.14%) - e/f (13.94%) -	cla (39.11%) + co (37.63%) - e/f (17.89%) -	cla (46.13%) + e/f (30.61%) - co (19.39%) -
Comparisons		UC *vs.* AMX1/ Cu1	UC *vs.* AMX2/Cu2	UC *vs.* AMX1/Cu2	UC *vs.* AMX2/Cu1
Trophic groups	ANOSIM	AD = 8.79% *R*-statistics = 1 *p* = 0.04	AD = 63.26% *R*-statistics = 1 *p* = 0.01	AD = 9.23% *R*-statistics = 0.1 *p* = 0.01	AD = 26.54% *R*-statistics = 0.667 *p* = 0.01
	SIMPER	1A (47.47%) -	1B (35.30%) - 2B (30.41%) +	2B (37.19%) + 1A (24.30%) -	2B (40.33%) +
Tail shapes	ANOSIM	AD = 12.66% *R*-statistics = 1 *p* = 0.01	AD = 26.37% *R*-statistics = 1 *p* = 0.03	AD = 6.51% *R*-statistics = 1 *p* = 0.01	AD = 24.60% *R*-statistics = 0.667 *p* = 0.01
	SIMPER	cla (45.99%) + e/f (35.44%) - co (13.48%) -	cla (50.00%) + co (22.44%) - e/f (22.21%) -	co (31.61%) - cla (26.19%) + e/f (25.76%) - s/r (16.44%) -	cla (49.77%) + e/f (24.07%) elim co (22.37%) -

Table 6. Binding affinities and closest interacting residues of amoxicillin with Germline Development Protein 3 (GLD-3) and Sex-Determining Protein (SDP) of *Caenorhabditis elegans*.

Entry	Affinity (kcal/mol)	RMSD (lower-upper)	Interacting Residues (distance, Å)	Closest Interacting Residue
GLD-3	−6.4	0.0-36.10	**Conventional H-Bond:** ILE107 (2.682), GLU108 (2.962), GLU108 (1.994), THR115 (2.189) **π-Alkyl:** ARG113	GLU108:O
SDP	−7.7	0.0-32.39	**Conventional H-Bond:** LYS82 (2.711), LYS82 (2.711), LYS82 (2.935), GLN175 (1.875), ASP166 (2.118), GLN78 (2.263)	GLN175:HE21