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2 **A new diatom-based multimetric index (MMI-D) for ecological health**
3 **monitoring in the Tropical Rift Valley Lake(Lake Hawassa, Ethiopia)**

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11 **Abstract**

12 *Multimetric assessment is one of the important tools for diagnosing, detecting and measuring the*
13 *level of impairments of ecosystem function in lentic ecosystem. It also provides detection*
14 *capability over a broader range and nature of stressors and gives a more complete picture of the*
15 *ecological conditions than single metrics and biological indicators. A diatom-based multimetric*
16 *index (MMI-D) was developed to evaluate the ecological-health of Lake Hawassa.*
17 *Physicochemical and benthic diatom sampling was done at nine sites with different degrees of*
18 *human disturbance along the lakeshore area from February to November 2015 and 2016. A priori*
19 *classification of lake segments into minimally disturbed (three sites), moderately disturbed (three*
20 *sites) and highly disturbed (three sites) was done by clustering sampling sites based on percentage*
21 *disturbance score (PDS). From 24 diatom candidate-metrics, only 10 were chosen as core metrics*
22 *for the development of MMI-D based on redundancy analysis, reaction to environmental*
23 *conditions, percent discriminatory efficiency (%DE), and box-plots. The newly established MMI-*
24 *D index clearly distinguished between reference and non-reference sites, as well as between the*
25 *lake's three clusters. The MMI-D index's performance was validated using independent data sets*
26 *from Lakes Hawassa and Ziway, and it demonstrated the best capability for discrimination*
27 *between different disturbance levels. MMI-D TSLS regression analysis revealed an inverse but*
28 *robust connection with PDS, indicating its responsiveness to Lake Hawassa habitat quality*
29 *degradation (n=9, R²=0.921, P=0.000). The MMI-D index revealed a high %DE (95.1%) and a*
30 *negative but significant connection with nutrients, total suspended solids (TSS), and turbidity*
31 *(R²>0.6; P<0.05). Generally, it can be concluded that this index is a powerful tool that could*
32 *assist end-users by providing a practical method for measuring the ecological quality of L.*
33 *Hawassa.*

34

35 **Keywords:** benthic diatoms, ecological quality, Lake Ziway, redundancy analysis, validation

36

37 **Introduction**

38 Lake Hawassa is one of the most threatened Rift Valley Lake because of human pressure related
39 to adverse watershed land use, urban development, and expansion of industries. For example,
40 around the 1980s, there were no observed pollution signs on adjacent sites of the lake to Hawassa
41 City since there were few recreational activities and urban development (Kibret 1985). Nowadays,
42 recessional farming, deforestation, urbanization, recreational activities and industrial expansion
43 are among the stressors type that significantly contributes to the observed change of the lake water
44 quality. As a result of the effect of these multiple stressors, the lake ecology has degraded in terms
45 of its physical (like modified shoreline, less of riparian vegetation cover, lots of manmade
46 structures in and around littoral zone), and chemical (like increasing concentration of nutrients)
47 (Wondmagegn 2019) and high bioaccumulation load of trace metals (Nigussie et al. 2010)). In
48 addition, there was also deterioration of biological quality (like dominancy of pollution tolerant
49 macroinvertebrate assemblages (Aklilu 2011).

50 Lakes of sub-Saharan Africa including Lake Hawassa are utilized for a variety of purposes that are
51 uncommon or unseen in developed temperate countries, like waste disposal, laundry washing,
52 cattle watering, and personal hygiene (Wondmagegn and Mengistou 2023). According to Revenga
53 et al. (2005) these activities have caused a decline in the overall ecological functioning, which has
54 altered in species abundance and richness of biological communities (Strayer 2006). Such changes
55 in benthic organisms in response to human pressures has been reported as effective tools to monitor
56 the biological integrity East African waterbodies (Masese et al. 2013a). Besides, Odountan et al.
57 (2019) recently suggested that benthic indices and metrics are advocated for the purpose of
58 biomonitoring of lakes in West Africa and developing countries. Therefore, to have best
59 management practices and to assure the sustainable function of the lake ecosystem, biological
60 monitoring uses aquatic organisms is important to monitor changes in chemical and physical
61 components of the aquatic environment. It can enhance the ability to identify the level of
62 degradation as well as the actions to take (Masese et al. 2013b; Stribling and Dressing 2015a;
63 Stribling and Dressing 2015b).

64 In earlier times, indicator organisms (metrics) were used for biomonitoring practice based on their
65 response to human perturbation in their community structure. Later, ecologists developed single
66 biotic index to get better description on the human disturbance to aquatic ecology than the indicator
67 organisms (Barbour et al. 1996). In recent years, using single biotic index is being replaced by
68 multimetric biotic index since single biotic index can respond to limited stressors which may
69 affect the accuracy of the assessment (Wang et al. 2015). Thus, the multimetric approach is more
70 robust water quality assessor and indicates ecosystem integrity, and provide opportunity to respond
71 for different stressors type at a time (Schoolmaster et al. 2012; Wang et al. 2015). This method
72 uses an aggregation of individual community metrics that comprise benthic biological elements
73 for the development of a single composite multimetric index (De la Rey et al. 2004) which are
74 recommended to use lakes suffered with multiple stress like that of Lake Hawassa (Wondmagegn
75 and Mengistou 2020). This can potentially reflect multiple effects of human impact on the structure
76 and function of aquatic ecosystem (Barbour et al. 1999; Menetrey et al. 2011) and is based on
77 comparing the biological metrics from minimally disturbed to highly disturbed sites of the water
78 body (Stoddard et al. 2006; Whittier et al. 2007).

79 The application of diatom based multimetric index in lake biomonitoring is quite recent and only
80 few studies have been recorded from tropical regions (Phiri et al. 2007; Wang et al. 2015; Chen et
81 al. 2017) but not Ethiopian lakes, including Lake Hawassa. Since the water quality of Lake
82 Hawassa has been affected by a number of stressors, a multimetric approach is highly
83 recommended (Wondmagegn et al. 2019) to obtain accurate results of the response diatoms to the
84 potential stressors of the littoral regions of the lake. Therefore, the objective of this study was to
85 develop a diatom base multimetric index of biotic integrity of L. Hawassa (MMI-D) and validate
86 its capability of discriminating reference from non-reference sites of the lake.

87 **Materials and Methods**

88 *Description of the study area*

89 Lake Hawassa, lies 275 km south of Addis Ababa, in the Main Ethiopian Rift (MER), surface
90 elevation 1,686 m asl ($6^{\circ}33' - 7^{\circ}33' \text{ N}$ and $38^{\circ}22' - 38^{\circ}29' \text{ E}$; **Figure 1**) (Welcome 1972). It has no
91 visible outlet, however, a UN Geothermal Survey has suggested that there may be groundwater
92 flow away from the lake on the south-west and north sides, which may account for a major loss of

93 water. This outflow could moreover be used to explain the relatively low alkalinity of Hawassa
94 compared with either saline Lake Shala or Lake Abiyata, both of which are also terminal lakes
95 (Makin et al. 1975).

96 Tikur Wuha, the only perennial river feeding the lake drains the vast swamps of Wendo Genet
97 area, which in itself drains the highlands on the east. The surface area of the lake is about 92 km²
98 (Makin et al. 1975), 16 km long, up to 8 km wide, and it has an estimated volume of 1.3 billion
99 m³. The maximum and mean depth of the lake is about 22 m and 11 m, respectively.

100 It is ecologically very important and is home to eight species of fish (Dadebo 2000; Tekle-Giorgis
101 et al. 2017), Pelicans, Storks, Herons, Hammerkops, Sea Eagles and Kingfishers. There is a small-
102 scale fish market on the shore at Amora Gedel. The lake also supports large mammals including
103 hippopotamus (Wondmagegn et al. 2019).

104 *Sampling site selection*

105 The sampling sites were selected according to their exposure to anthropogenic activities by
106 computing its habitat quality through percent disturbance score (PDS; **Table 1**) (Fig. 1). So, KW1,
107 KW2 and WSH were categorized as reference groups whereas, the remaining sites (such as
108 MK, TW1, TW2 of moderately disturbed sites and WR, RH1 and AG1 of Highly disturbed sites)
109 were classified as non-reference groups (**Table 1**) according to Wondmagegn et al. (2019). All the
110 physicochemical and the biological sampling was done on these categorized sites with three replications.

111 *Sampling of environmental parameters*

112 A combined portable HQ40D multimeter was used to measure *in situ* physicochemical parameters
113 such as temperature (T), pH, conductivity (EC) and dissolved oxygen (DO). The turbidity was
114 measured with an OAKTON turbidimeter (T-100). NO₂⁻, NO₃⁻, NH₄⁺, soluble reactive phosphate
115 (SRP) and total phosphorus (TP) were analysed spectrophotometrically in the Limnological
116 Laboratory of Addis Ababa University. Nitrate was analysed with sodium salicylate method
117 (Robarge et al. 1983), ammonium with indo-phenol blue (APHA 1995), and soluble reactive
118 phosphate (SRP) with ascorbic acid method (APHA 1999). Nitrite concentration was determined
119 by the reaction between sulfanilamide and N-naphthyl-(1)- ethylenediamine dihydrochloride
120 (APHA 1995). Total phosphorus (TP) and silica (SiO₂) were determined using persulfate digestion
121 method and molybdosilicate method (APHA 1999), respectively.

122 The Chlorophyll *a* concentration was estimated according to the method of Talling and D. (1963).
123 From each site, 200–500 ml of the lake water was filtered through Whatman GF/F filters. The
124 filters were folded with aluminium foil, labeled and transported to the laboratory in an icebox and
125 stored for not longer than one day. Pigments were ground and extracted in 90% acetone. After
126 grinding, the algal material was centrifuged. Then, the extract was decanted into 5 ml cuvette and
127 the absorbance of Chlorophyll *a* was measured spectrophotometrically at wavelengths of 665 nm
128 and 750 nm, respectively. The total suspended solids (TSS) samples were filtered using Whatman
129 GF/F filters and analyzed following Wetzel and Likens (2000)

130 *Sampling of benthic diatoms*

131 Diatoms were scraped from cobbles and macrophytes in the littoral areas of Lake Hawassa at the
132 maximum of 1 m depth. Sampling was conducted by taking cobbles (five stones with upper surface
133 areas of cobble ~25 cm²) and macrophytes (five random stems of macrophytes with 5 cm length)
134 from the lake shore (King et al. 2005; Martin and Fernandez 2012). Small amounts of lake water
135 (approximately 50 ml) were poured into a tray the biofilm from the upper surface of each cobble
136 and the macrophyte was removed by scrubbing vigorously with a toothbrush. The toothbrush was
137 rinsed regularly with lake water. Finally, the suspension was poured into a labeled 150 ml plastic
138 bottle. Before pouring, the suspension was swirled in the tray so that any settled particles were re-
139 suspended. Then, the diatom samples were preserved with 70% ethanol.

140 A 5-10 ml aliquot of each sample was taken from the bottle and homogenized by shaking. Diatom
141 samples were treated with concentrated sulfuric acid and potassium dichromate (Patrick and
142 Reimer 1966). Then, a drop of cleaned diatom samples were taken and dropped onto a microscopic
143 slide and placed on hot plate. After it had dried, a permanent slide was prepared by adding
144 Naphrax® (refractive index of 1.73) to the coverslip and placing the latter into the dried slide.
145 Diatom frustules were examined with Carl Zeiss Axioskop light microscope at 1000 x
146 magnification, with oil immersion objective, using bright-field illumination with a green filter to
147 increase the contrast in the laboratory of Environmental Centre of Lancaster University, UK.

148 Identification of diatom species was made by standard identification keys, manuals and
149 publications of Van Meel (1954); Gasse (1986); Krammer and Lange-Bertalot (1986 and 1988
150 and 1991b and 1991a); Kelly (2000); Taylor et al. (2007a); Taylor et al. (2007b); (Taylor and

151 Cocquyt 2016). For each slide, 500 valves were counted and relative abundance, as percentage,
152 was calculated for each species.

153

154 *Diatom indices/metric calculation*

155 OMNIDIA software version 6.1 was employed to calculate the diatom indices/metrics listed in
156 **Table 2** (Lecointe et al. 1993). Four diatoms (i.e. RAT, RRT, PRAT and PRRT; **Table 2**) reference
157 based metrics were included as candidate metrics and calculated using Microsoft excel. The
158 development of the MMI-D index was based on three *a priori* clustered sites, namely, C1-
159 minimally disturbed sites (KW1, KW2 and WSH), C2- moderately disturbed sites (MK, TW1 and
160 TW2) and C3-highly disturbed sites (WR, RH1 and AG1).

161 *Selection and removal of redundant metrics*

162 A total of 24 diatom candidate metrics (17 of them were published in Wondmagegn et al. (2019);
163 **Table 2**) representing various aspects of the diatom communities related to family richness,
164 taxonomic composition, tolerance measures, biotic indices and others (Kelly and Whitton 1995;
165 Rott et al. 1999; Lecointe et al. 2003) were compiled.

166 The listed candidate metrics/indices were used for the selection of the potential/core metric which
167 were incorporated in the development of multimetric index of biotic integrity of Lake Hawassa
168 (MMI-D). The procedure for selecting the potential metric was done according to Barbour et al.
169 (1999), with some modification. The selection was done based on the metrics response to
170 physicochemical parameters and with the relationship between themselves and their ability to
171 characterize the reference and non-reference sites. Metrics which significantly correlated with
172 physicochemical variables were selected for the next analysis. Redundancy analysis was employed
173 to identify the pair of metrics with a significant correlation (i.e., correlation coefficient ≥ 0.7)
174 (Ofenböck et al. 2004; Hering et al. 2006). From the redundant metrics, the one which strongly
175 correlated with the physicochemical variables was considered for further analysis and the others
176 were rejected.

177 Percent discriminatory efficiency (%DE) of each metric was calculated to identify the most
178 suitable metrics having high % DE (usually greater than or equal to 50%) as was used in

179 Wondmagegn et al. (2019) and Wondmagegn and Mengistou (2023) . Besides this, for diatom
180 indices selection, percent inclusion of the diatom species in index calculation was also considered
181 as an important criterion in which <50% inclusion was excluded for further analysis (Wondmagegn
182 et al. 2019). The distribution of each metrics between the reference and non-reference sites was
183 visualized using Box and Whisker plots. The sensitivity of the metrics was examined based on
184 their interquartile overlap degree according to the method outlined in Barbour et al. (1996).

185 *Scoring of metrics*

186 Using the discrete type of scoring system, calculated metric values were converted (normalized)
187 to metric scores of 5, 3 or 1 depending on their proximity to the optimal values as used in
188 Wondmagegn and Mengistou (2023). Metrics whose values decreased with the increase of
189 disturbance (positive metric) 5, 3 and 1 scoring was used, and the reverse scoring for negative
190 metrics. For instance, positive metrics values above 75th percentiles were scored as 5. Metric values
191 between and including the 75th and 25th percentiles were scored as 3, and all metric values below
192 the 25th percentile were scored as 1 (Barbour et al. 1996; Wang et al. 2005).

193 *Development of multimetric index of biotic integrity for Lake Hawassa (MMI-D)*

194 The scored values of each potential metric value were combined into a multimetric diatom index
195 (MMI-D) by summing up the score of each individual metric. The possible maximum and
196 minimum MMI-D index values were calculated (maximum value= total number of selected metric
197 multiplied by 5, and minimum value= total number of selected metric multiplied by 1) and divided
198 into quartile ranges to have four quality classes. Thus, the highest score classified as very good
199 quality and the lowest score as poor quality. Box and Whisker plots were also used to visualize
200 MMI-D index's distribution between the reference and non-reference sites and to test its potential
201 to discriminate the minimally disturbed sites from the moderately and highly disturbed sites of
202 Lake Hawassa.

203 *Ecological quality ratio (EQR)*

204 The ecological quality ratio (EQR) of each sampling station was used for the purpose of
205 classification of ecological status (Wondmagegn and Mengistou 2023). EQR was calculated by
206 dividing MMI-D values of each site with the median MMI-D values of reference sites. The ratio

207 is expressed as a numerical value usually between zero and one. Then the 90th percentile of the
208 reference site of the EQR values was used to classify into five ecological classes. Generally, as the
209 value of EQR becomes close to one, it is considered as high ecological status and as its values
210 approaches to zero, it is considered as bad ecological status (EQR 2007; Delgado et al. 2010).

211 *Validation of the MMI-D*

212 The validation of MMI-D index was conducted using independent data sets which were not
213 incorporated in the MMI-D index development of L. Hawassa. One of the independent data set
214 was taken from the reference and non-reference sites of Lake Hawassa. In addition, it was also
215 validated using the independent data set from L. Ziway with box and whisker plot of Sigma
216 Software version 10.0. The independent data set of Lake Ziway was taken form unpublished data
217 of Abnet Woldesenbet. Two-stage Least Squares (TSLS) regression analysis was also employed
218 to test the relationship between the MMI-D index and percent disturbance score (PDS) in the SPSS
219 package version 20. Principal component analysis (PCA) was also used to visualize the capability
220 of MMI-D index's distribution between the reference and non-reference sites of Lake Hawassa.

221 *Data analysis*

222 OMNIDIA software version 6.1 was employed to calculate the diatom indices/metric. Spearman
223 rank correlation was used to check relationships of candidate metrics with themselves and with
224 environmental parameters, in order to select the core metrics. Two-stage least squares (TSLS)
225 regression analysis was also employed to test relationship between the MMI-D index and percent
226 disturbance score (PDS). The above analyses were done with Statistical Package for Social Science
227 Students (SPSS Inc., software version 20.0). Box and Whisker plot and Principal components
228 analysis (PCA) were employed to show the discriminatory potential of the multimetric index
229 (MMI-D) among the reference and test sites of Lake Hawassa using Sigma-plot version 15.0 and
230 PAST 3.15 software programmes, respectively. The validation of the potential of MMI-D index
231 with independent data set of L. Ziway was checked with Box and Whisker figures using Sigma-
232 plot version 10.0.

233 **Result**

234 *Metric Selection*

235 Metric selection was done based on percent inclusion of the diatom species in index calculation
236 (the more species are included in index calculation; the more efficient index is to explain the
237 ecology). In addition, their discrimination efficiency, correlation, and response to environmental
238 parameter were checked (**Table 2&3**). Therefore, IDAP, P.SI, WAT, DES, LOBO, PDI, and P.TI
239 indices were excluded from selection due to <50% inclusion of the diatom species in the index
240 calculation using OMNIDIA software. These indices did not also exhibit a significant correlation
241 with ecologically important physicochemical variables such as TP, SRP and Nitrate (**Table 3**).
242 IBD, IPS, and CEE were not also included in metrics selection because of having a high correlation
243 with SHE, IDG and TDI.

244 Thus, indices those which had greater than 50% discrimination efficiencies, $\geq 50\%$ species
245 inclusion, lack redundancy and showed a significant correlation with most of physicochemical
246 variables (EPID, SHE, SLA, IDG, TDI, ROTT, TDIL and LDTI2) were included in metrics
247 selection (**Table 3**). From the four reference taxa-based metrics, RATD and PRATD were selected
248 due to their high discriminatory efficiency for the multimetric index (MMI-D) development (**Table**
249 **2& 3**). These metrics did not exhibit a complete overlap on their interquartile and had potential to
250 characterize the reference sites and non-reference sites as illustrated on **Fig. 2**.

251 *Developments of multi-metric index of L. Hawassa (MMI-D)*

252 From the list of 24 diatom candidate metrics, 10 diatom metrics were selected, based on their
253 response to the different level of disturbances, discriminatory power between the reference and
254 test sites, and correlation with physicochemical parameters. The selected metrics were used for the
255 development of multimetric index of biotic integrity of L. Hawassa (MMI-D; **Table 4**). The MMI-
256 D used a 5, 3 or 1 discrete type scoring system to normalize the metric value for positive metric
257 using 75th and 25th percentiles of the metric values and the reverse for negative metrics. For positive
258 metrics, metric values above 75th percentiles were scored as 5. Metric values between and
259 including the 25th and 75th percentiles were scored as 3, and all metric values below the 25th
260 percentile were scored as 1 (**Table 5**). The sum of the total score of each site was considered as the
261 MMI-D values. The maximum and minimum possible ranges of the MMI-D values were 50 and

262 10, respectively. These MMI-D values were divided into quartile ranges. So, the MMI-D range
263 values 41-50, 31-40, 21-30, and 10-20 were classified as very good, good, fair and poor quality,
264 respectively.

265 Therefore, the current study showed that the multimetric index (MMI-D) had a potential to
266 discriminate clearly the reference site and the non-reference and between the three-disturbance
267 level (minimal, moderate and high disturbance level) of the clustered sampling stations of the lake
268 (**Fig. 3**). It put the minimally disturbed sites (C1) into very good to good categories (KW1, KW2,
269 and WSH), moderately disturbed sites (C2) into fair (MK, TW1 and TW2) and highly disturbed
270 sites (C3) into poor categories (**Table 6**).

271 The discrimination capacity of the MMI-D index between reference and non-reference sites was
272 tested by Box and Whisker plots within the comparison of the previously classification of sampling
273 stations of L. Hawassa. Thus, the MMI-D clearly discriminated the reference sites from the non-
274 reference (test) sites (**Fig. 3a**). It also showed a demarcation potential between the three clusters
275 such as C1 (minimally disturbed), C2 (moderately disturbed) and C3 (highly disturbed sites) of
276 the lake (**Fig. 3b**).

277 *Ecological quality ratio (EQR)*

278 The ecological quality ratio (EQR) was calculated and its range was between one and zero, but
279 there were values greater than one which were considered as one. The 90th percentile of the
280 reference site of the EQR values was 1. The sampling sites ecological status was classified based
281 on the EQR values with its range (**Table 7**).

282 The Ecological Quality Ratio potentially discriminated the reference and non-reference sites. It
283 characterized the minimally disturbed (C1) sites into high and good quality or reference condition
284 (KW1, KW2, and WSH), moderately disturbed sites into moderate quality and highly disturbed
285 sites into poor quality sites (**Table 8**). The Box and Whisker plots also showed that the EQR had
286 the efficiency to discriminate between reference and non-reference sites and the three clustered
287 sites (C1, C2 and C3; **Fig. 4**).

289 The efficiency of the MMI-D index to discriminate between reference and non-reference sites, and
290 between *a priori* classification of the sampling station (i.e. minimal, moderate and high disturbance
291 levels) were tested by Box and Whisker plots in previous section. Furthermore, to confirm the
292 suitability and robustness of this newly developed index (MMI-D index) it requires validation. The
293 validation of the MMI-D index was performed using independent data sets where the MMI-D
294 index was not based. Thus, the performance of the MMI-D index was tested in the new data sets
295 of Lake Hawassa and Lake Ziway. Three sites were taken from Lake Hawassa and subjected to
296 validation using the MMI-D index. Thus, the MMI-D classified one site as very good (MMI-D
297 value= 34), and the other two sites as fair (MMI-D value= 28 and 28) which coincided with prior
298 classification of these sites.

299 The developed MMI-D also discriminated L. Ziway's reference and test sites as shown in **Fig. 5**.
300 It showed good potential of characterizing the L. Ziway clustered sites. For example, all the three
301 reference sites (C1) were classified as very good quality; from three moderately disturbed sites
302 (C2) all of the sites were characterized as fair. The three highly disturbed sites (C3) of the lake
303 were also classified as fair to poor category. The principal component analysis (PCA) also clearly
304 explained 96.85% of the variation between the reference site and non-reference/test site of Lake
305 Hawassa with both the first and the second axis (**Fig. 6**). This confirms the potential of the MMI-
306 D index to discriminate between reference and non-reference sites of Lake Hawassa.

307 The two-stage least squares (TSLS) regression analysis of MMI-D showed an inverse but strong
308 relationship with percent disturbance score (PDS) which demonstrates the MMI-D index
309 responsiveness to the habitat quality degradation of Lake Hawassa ($n=9$, $R^2=0.921$, $P=0.000$; **Fig.**
310 **7**). The MMI-D index also showed significant positive response to DO, and negative but
311 significant correlation with temperature, nutrients, TSS and turbidity and had high percent
312 discriminatory efficiency (%DE= 95.1; **Table 9**).

313 **Discussion**

314 Lake Hawassa has been affected by multi-type stressors which come from different sources (more
315 often from non-point sources), the need of multimetric index development is a mandatory practice
316 to quantify the ecological status of the sampling stations that have suffered from human induced

317 level of disturbances. The multimetric index of biotic integrity of L. Hawassa (MMI-D) was
318 developed in order to harvest these benefits.

319 The multimetric index of biotic integrity of L. Hawassa (MMI-D) showed a clear demarcation
320 between the reference and non-reference/ test sites (**Table 6; Fig. 3**). It also placed the minimally
321 disturbed sites (C1) as very good to good categories (KW1, KW2 and WSH), moderately disturbed
322 (MK, TW1 and TW2) sites into fair and highly disturbed (WR, RH1 and AG1) sites into poor
323 quality (**Table 6**). Hence, the MMI-D index showed a robust result able to discriminate the three
324 clusters of the sampling stations of the lake as previously classified. Besides, the ecological quality
325 ratio (EQR) explained the status of the sampling sites in-terms of their ecological condition (**Table**
326 **8**) as it is believed that EQR is an ecological expression of the MMI-D index (Lepistö et al. 2004;
327 Wells et al. 2007; Gabriels et al. 2010).

328 Generally, the MMI-D index and the EQR showed robust results that clearly discriminate sampling
329 stations into their ecological quality level as very good, fair and poor quality. Hence, conservation
330 of very good quality sites and rehabilitation of the fair and poor-quality sites are important
331 recommendations that can be drawn from the current result.

332 The suitability and robustness of a newly developed multimetric index (MMI-D) requires
333 validation which is an effective method for evaluating the lake condition to recommend future use
334 of the MMI-D, effective restoration and conservation methods and research gaps (Jun et al. 2012).
335 Validation of MMI-D using independent data sets is an appreciable method that indicates how well
336 the multimetric index would be expected to work with sampling sites and gives good insight to
337 scale up and use for other lakes in the ecoregion (Lunde and Resh 2012; Villamarín et al. 2013).
338 The potential use of MMI-D was confirmed by the discrimination efficiency of the independent
339 data set taken from Lake Hawassa and L. Ziway, which coincided with prior classification of these
340 sites. Thus, the validation of the MMI-D index showed its potential to be used in the biomonitoring
341 of the initial lake and lakes which are situated in the same ecoregion of Lake Hawassa, namely
342 Northern Eastern Rift (Thieme et al. 2005).

343 Furthermore, the MMI-D was further validated using physicochemical parameters, PCA, and its
344 relationship with disturbance score (PDS) that showed the responsiveness of the MMI-D with
345 different levels of degradation which were already observed on the reference and non-reference

346 sites. Thus, the significant inverse relationship between MMI-D and PDS represents a strong
347 responsiveness of the MMI-D index to the lake habitat quality levels (PDS: $n=9$, $R^2=0.921$,
348 $P<0.01$, **Fig. 7**). Similar phenomenon was observed on the study of multimetric index of Lake
349 Kariba of Africa (Phiri et al. 2007), lakes of USA (Stevenson et al. 2013) and Lake Dongting
350 (Wang et al. 2015). Since the MMI-D index is considered as a positive metric, its value decreased
351 against the habitat disturbance (**Fig. 7**) as was observed on wetlands of California (Lunde and Resh
352 2012). This wetland report showed that a higher multimetric index scores was considered as signal
353 of a less disturbed environment, while lower multimetric index scores indicated highly disturbed
354 habitat. MMI-D index showed a better potential than that of the single metrics of diatoms tested in
355 Wondmagegn et al. (2019). Similar potential was also observed on the multimetric developments
356 of Alaska's biomonitoring practice with high precision and high discrimination efficiency
357 (Bouchard et al. 2004).

358 In addition, the MMI-D index also showed significant positive response to DO, and negative but
359 significant correlation with temperature, nutrients, TSS and turbidity (**Table 9**). Similar response
360 of the MMI index to nutrient concentration was observed in lakes of USA (Stevenson et al. 2013),
361 Lithuanian lakes (Šidagytė et al. 2013) and lakes in Flanders, Belgium (Gabriels et al. 2010). The
362 negative response of the MMI-D index to nutrients, TSS and turbidity corresponds with what can
363 be expected for stress-related variables (Gabriels et al. 2010). This indicates that the MMI-D index
364 was suitable for ecological quality assessment of Lake Hawassa.

365 The overall finding of the current research indicated that such multimetric approach has significant
366 advantage when the water body is exposed to multiple stressor types not usually counted (Everard
367 et al. 2011). The index can also reduce the independent metric prediction problem and maximize
368 the efficiency to express the not-visualized and unmeasured disturbance of segments of the lake
369 ecosystem. As noted by Schoolmaster et al. (2012), when there is a problem of understanding the
370 exact cause of degradation of water quality, the multimetric approach is most effective. The
371 discrimination power of the MMI-D index between different levels of human disturbance showed
372 the effectiveness of multimetric approach.

373 In addition, the benefit of such MMI-D development to specific lake would also increase the
374 performance of the multimetric index by reducing natural variability (like in geology, soils,

375 landscapes, climate, and water chemistry) among sites since it is difficult to distinguish the effect
376 of natural variability from human induced variability if it is applied across large spatial scale
377 (Stevenson et al. 2013). Furthermore, the MMI-D index clearly discriminated the reference and
378 non-reference sites of Lake Hawassa and showed similar performance on Lake Ziway (within the
379 same ecoregion); thus, it can be recommended for application to biomonitoring activity in other
380 lakes of the same ecoregion.

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390 **Declaration of competing interest**

391 The authors declare that they have no known competing financial interests or personal
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