

1 **Application of soil quality indices to assess the status of agricultural**
2 **soils irrigated with treated wastewaters**

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8 **Abstract**

9 The supply of water is limited in some parts of the Mediterranean region, such as
10 southeastern Spain. The use of treated wastewater for the irrigation of agricultural soils
11 is an alternative to using better-quality water, especially in semi-arid regions. On the
12 other hand, this practice can modify some soil properties, change their relationships and
13 influence soil quality. In this work two soil quality indices were used to evaluate the
14 effects of irrigation with treated wastewater in soils. The indices were developed
15 studying different soil properties in undisturbed soils in SE Spain, and the relationships
16 between soil parameters were established using multiple linear regressions. These
17 indices represent the balance reached among properties in “steady state” soils. This
18 study was carried out in four study sites from SE Spain irrigated with wastewater,
19 including four study sites. The results showed slight changes in some soil properties as a
20 consequence of irrigation with wastewater, the obtained levels not being dangerous for
21 agricultural soils, and in some cases they could be considered as positive from an
22 agronomical point of view. In one of the study sites, and as a consequence of the low
23 quality wastewater used, a relevant increase in soil organic matter content was observed,
24 as well as modifications in most of the soil properties. The application of soil quality
25 indices indicated that all the soils of study sites are in a state of disequilibrium regarding
26 the relationships between properties independent of the type of water used. However,

27 there were no relevant differences in the soil quality indices between soils irrigated with
28 wastewater with respect to their control sites for all except one of the sites, which
29 corresponds to the site where low quality wastewater was used.

30 **Keywords:**

31 Soil quality index, Wastewater, Soil organic carbon, Multiple lineal regression models,
32 Mediterranean soils.

33 **1. Introduction**

34 In the southeastern region of Spain, the accessibility to groundwater is low due
35 to the climate, rapid development of agricultural and touristic activities and fast
36 expansion of the industrial sector which have produced an over-exploitation of aquifers.
37 For this reason, freshwater availability is a current limiting factor in Mediterranean
38 areas, and it is necessary to find alternatives to satisfy the strong water demand.
39 Consequently, any activities taken to combat water scarcity challenges should be
40 sustainable and should not reduce the natural resources or damage the environment.

41 The application of wastewater to irrigate agricultural soils is not a new practice
42 (Day et al., 1974; Weber et al., 1996; Brar et al., 2002; Mohammad and Mazahreh,
43 2003; Pedrero et al., 2010). The reuse of industrial and urban wastewater has increased
44 in many places principally because of demand in the agriculture sector. One of the best
45 options for using treated wastewater can be the irrigation of agricultural soils, which
46 allows the retention of a large amount of fresh water for other purposes (Pescod et al.,
47 1992). Although the idea is currently receiving greater consideration because of the
48 global water crisis, this reuse has been practiced all over the world (Asano, 1991;
49 Keremane and Mckay, 2008). Substitution of freshwater by treated wastewater, richer in
50 nutrients, is a key conservation strategy contributing to agricultural production (Rattan
51 et al., 2005; Lin et al., 2006; Mekki et al., 2006; Rosabal et al., 2007).

52 Another environmental problem in the Mediterranean area is soil degradation. In
53 some cases this degradation is due to the kind of agricultural irrigation applied, in many
54 cases, using low-quality waters (waters with a very low degree of depuration and
55 consequently with a high salts content and in some cases contaminant compounds).
56 Additionally, under semi-arid conditions, where the precipitation is less than the
57 potential evaporation, the soils are prone to organic matter loss (Anderson, 2003),
58 because under these climate conditions the high temperatures produces a fast organic
59 matter oxidation, and due to the scarcity of rainfall, the vegetation cover is very low and
60 therefore there are low inputs of organic matter into the soil. The alternative use of
61 treated wastewater, could offer an additional source of organic matter and nutrients, the
62 recovery of soil properties and increasing the storage of organic carbon in the soil in the
63 medium-term (Burns et al., 1985; Friedel et al., 2000).

64 However these practices may have adverse effects on soil quality. Agricultural
65 management has been considered as one of the greatest causes of soil degradation
66 (Kieft, 1994). Continuous soil tillage and the incorporation of organic residues or
67 fertilization could provoke alteration in some soil properties: organic matter, aggregate
68 stability and enzyme activity (Caravaca et al., 2002; Gardi et al., 2002). Any disturbance
69 in soil properties is usually accompanied by a loss of soil quality (Zornoza et al.,
70 2007a). The evaluation of soil quality could be helpful to assess the level of disturbance
71 in agricultural soils and useful in deciding the best alternative to having an adequate
72 crop production preserving the soil quality (Karlen et al., 1997).

73 In the last years, there has been a growing trend in publications based on the use
74 of biological and biochemical properties in evaluating soil quality due to the high
75 sensibility of these properties (Trasar-Cepeda et al., 1998; Caldwell et al., 1999;
76 Badiane et al., 2001; Filip, 2002; Salamanca et al., 2002; Wick et al., 2002; Ruf et al.,

77 2003; Bastida et al., 2008) and based on an integration of physical, chemical and
78 biological properties, owing to the close interaction among these properties in soil
79 (Wander and Bollero, 1999; Andrews and Carroll, 2001; Aon et al., 2001; Chapman et
80 al., 2003; Sparling et al., 2004).

81 Multiple linear regressions have also been successfully used as a method to
82 choose the indicators to form part of the quality index and also as a tool to create
83 algorithms to evaluate soil quality (Trasar-Cepeda et al., 1998; Emmerling and
84 Udelhoven, 2002; Lentzsch et al., 2005). With this methodology, a variable is calculated
85 by linear combination of others. Furthermore, only the variables that significantly
86 explain the highest variance in the predicted variable are chosen. Thus, this method is
87 also useful in reducing the number of soil indicators forming part of the index
88 (Caravaca et al., 2002).

89 Although, there are not previous studies using soil quality indices to assess the
90 use of wastewater for irrigation in agriculture, there are some that used soil quality
91 indices to evaluate the effect of soil contamination with industrial and municipal
92 wastes, organic fertilisation or irrigation with poor quality water under different crops
93 (Puglisi et al., 2005). We used two indices, developed by Zornoza et al. (2007a), to
94 evaluate the effects of medium and long-term irrigation with treated wastewater on the
95 soil quality of some agricultural soils of SE Spain.

96

97

98 **2. Materials and methods**

99 *2.1. Study sites and soil sampling*

100 Three areas in the province of Alicante were selected for the study (figure 1),
101 called Alicante (A), Monforte del Cid (M) and Biar (B), according to their geographical
102 location. In the Biar area, two sites were sampled (B1 and B2). In all the four sites
103 wastewater was used for irrigation.

104 In table 1 the main characteristics of study sites are shown. Site A is agricultural
105 land near to Alicante city dedicated to oranges. In this site there are two plots, one that
106 has been irrigated with treated wastewater during 40 years (AR), and the other that has
107 been irrigated with fresh water during the same period (AC). Site M is orchard land
108 with a grape crop irrigated with treated wastewater during 20 years (MR) and its control
109 site was irrigated with fresh water for the same period of time (MC). In both areas
110 irrigation has been carried out by drip. Site B1 is agricultural land with a grape crop
111 irrigated using treated wastewater for two years (B1R) and control irrigated with fresh
112 water (B1C), in this case the irrigation method used was flooding. Site B2 is land near
113 to a wastewater treatment plant, where there are two plots, one that has been irrigated
114 with very low quality wastewater for a long-term (20 years) (B2R) and control plot
115 (B2C) close to this is an area dedicated to almond crop with similar soil conditions and
116 not irrigated with wastewater.

117 B2R is 2 ha plot that has been used as a ‘green filter’ during approximately 20
118 years and was irrigated with ‘low quality’ wastewaters, which had a very low degree of
119 depuration. Analytical data from 1994 to 2000 indicates very high levels of organic load
120 in the wastewater (In table 2 the mean values from monthly analytical data are shown.
121 Source: EPSAR, 2010).

122 Soil samplings were carried out in June 2010. Six samples per study site were
123 taken from the 0-5 cm depth (n=48). The samples were air-dried in the laboratory at
124 room temperature (~25°C) for a week. After that, they were sieved to 2 mm for
125 analyses, except for aggregate stability soil samples aliquots were sieved between 4-
126 0.25. For all analyses, two replicates per sample were used to reduce the analytical
127 error.

128 2.2. *Soil quality indices.*

129 Zornoza et al. (2007b) established two models for soil quality evaluation. Both
130 models were developed using multiple linear regressions between physical, chemical
131 and biochemical properties in undisturbed forest soils in SE Spain, representing the
132 relationship between soil parameters at “steady state”. The models were latter validated
133 using undisturbed and disturbed soils subjected to perturbations such as the use of land
134 for agriculture (Zornoza et al., 2008). Model 1, that explained 92% of the variance in
135 soil organic carbon (SOC) showed that the SOC can be calculated by the linear
136 combination of 6 physical, chemical and biochemical properties (acid phosphatase,
137 water holding capacity (WHC), electrical conductivity (EC), available phosphorus,
138 cation exchange capacity (CEC) and aggregate stability (AS)). Model 2 explained 89%
139 of the SOC variance, which can be calculated by means of 7 chemical and biochemical
140 properties (urease, phosphatase, and β -glucosidase activities, pH, EC, P and CEC). We
141 use the residual (difference between calculated SOC by models and real SOC measured
142 in laboratory) as soil quality indices. The soil will be equilibrated if residuals are near 0
143 or inside confidence intervals of the models (95%).

144 Model 2 is more sensitive owing to the high sensitivity of biochemical indicators
145 (enzyme activities) (Nannipieri et al., 1990; Dick et al., 1996; Van Brugger and
146 Semenov, 2000). Model 1 is less sensitive than Model 2, but suitable for assessing

147 severe states of degradation due to physical and chemical properties (Filip, 2002;
148 Reynolds et al., 2002).

149 Climatic factors have a strong influence on soil, for this reason, the models also
150 include the mean annual precipitation (Pm) of each study site as an independent variable
151 in the regressions, divided in two categories (Pm < 350 mm and Pm > 350 mm). The
152 selection of category was in function of Pm for each area (Table 1). Therefore, there are
153 two equations for both models, exposed below; the first formula (A and C) corresponds
154 to multiple linear regression for soils with Pm < 350 mm, in our case, Alicante and
155 Monforte sites. The second formula (B and D), adds a correction over the previous
156 formula for soils placed in sites with Pm > 350 mm, in this study both the sites located
157 in Biar. In these equations: SOC is expressed in g kg⁻¹, phosphatase and β-glucosidase
158 activities in μmol *p*-nitrofenol g⁻¹ h⁻¹, urease activity in μmol NH₄⁺ g⁻¹ h⁻¹, WHC and
159 AS in %, available phosphorus in mg kg⁻¹, EC in μS cm⁻¹ and CEC in cmol⁺ kg⁻¹.

160 In Model 1, SOC (transformed to Ln to achieve normality) can be calculated by
161 the following equations:

162 $\text{Ln (SOC)} = A$ (for Pm < 350 mm); or $\text{Ln (SOC)} = A + B$ (for Pm > 350 mm)

163 $A = 2.459 + 0.090$ (phosphatase) + 0.010 (WHC) + 0.001 (EC) - 0.009
164 (available phosphorus) + 0.012 (CEC) + 0.001 (AS)

165 $B = -0.138 - 0.007$ (WHC) + 0.059 (P) + 0.008 (AS)

166 This model explains 92% of the variance in SOC. The confidence interval (CI)
167 at 95% of the residuals distribution of the model ranged from -0.21 to 0.21.

168 With Model 2, SOC (transformed to Ln) can be calculated by following
169 equations:

170 $\text{Ln (SOC)} = C$ (for Pm < 350 mm); or $\text{Ln (SOC)} = C + D$ (for Pm > 350 mm)

171 $C = 5.527 + 0.150$ (phosphatase) - 0.064 (Urease) - 0.088 (β -glucosidase) - 0.291
172 (pH) + 0.001 (EC) + 0.028 (available phosphorus) + 0.028 (CEC)

173 $D = 0.037 + 0.208$ (β -glucosidase) - 0.015 (CEC)

174 This model explains 89% of the variance in SOC. The confidence interval (CI)
175 (at 95%) ranged from -0.23 to 0.23 (Zornoza et al., 2008).

176 Any disturbance in soil must be accompanied by the modification of its
177 properties and its equilibrium as observed in undisturbed forest soils. As a consequence,
178 SOC calculated by the models (SOCc) is no longer an accurate estimation of the actual
179 SOC determined in laboratory (SOCa). For a non-disturbed soil, in which these
180 properties are balanced with organic matter, the values of residuals are 0 (SOCc =
181 SOCa). Accordingly, as disturbing practices provoke a disruption of this equilibrium,
182 SOCc should be lower or higher than the actual SOC, and degraded soils should
183 generate residuals with values < or > 0. In addition, the more the degree of degradation
184 increases, the more the values of SOCc should differ from the values of SOCa. For this
185 reason, a soil quality index (SQI) was obtained by calculation of the model residuals:

186
$$SQI = \text{model residual} = SOCc - SOCa$$

187
$$SQI 1 = \text{model 1 residual} = SOCc - SOCa$$

188
$$SQI 2 = \text{model 2 residual} = SOCc - SOCa$$

189 These two models of SQI have been applied to the different sites of this study.
190 We hypothesised that soils with more sustainable management practices should result in
191 $SQI \approx 0$ (within the 95% CI of the residuals distribution of the models). On the contrary,
192 in soils with damaging tillage practices or recent changes in management, SQI should
193 be < or > 0.

194 *2.3. Analytical methods*

195 Soil pH was analysed in a 1:2.5 w/v, electrical conductivity (EC) in a 1:5 w/v,
196 texture determined by the Bouyoucos method (Gee and Bauder, 1986). Soil organic
197 carbon (SOC) was determined by Walkley and Black (1934). Available phosphorus was
198 determined by the Burriel-Hernando method (Díez, 1982). Water holding capacity
199 (WHC) was assayed by the method exposed by Forster (1995). Aggregate stability (AS)
200 was measured using the method of Roldán et al. (1994); this method examines the
201 proportion of aggregates that remain stable after a soil sample (sieved between 4-0.25
202 mm) is subjected to an artificial rainfall of known energy (270 J m^{-2}). Cation exchange
203 capacity (CEC) was measured by the method described by Roig et al. (1980). Urease
204 activity was measured according to the method of Nannipieri et al. (1980). Acid
205 phosphatase activity was assayed by the method of Tabatabai and Bremmer (1969). The
206 activity of β -glucosidase was determined according to Tabatabai (1982).

207 *2.4. Statistical analysis*

208 The fitting of the data to a normal distribution for all properties measure was
209 checked with the Kolmogorov-Smirnov test at $P < 0.05$. To compare the effect of
210 irrigation between the different types of waters, a T-Student test was developed at P
211 < 0.05 . All statistical analysis was performed with the SPSS program (Statistical
212 Program for the Social Sciences 18.0).

213 **3. Results**

214 *3.1. Soil properties*

215 Table 3 shows the soil physical, chemical and biochemical properties analysed
216 for every study site. The results show that there are some statistical differences although
217 not very significant in absolute values between soils irrigated with treated wastewater
218 and soils irrigated with fresh water from studied areas of Alicante, Monforte and Biar 1.
219 However, large differences were found in soils from Biar 2 due to the irrigation with

220 wastewater. In the Alicante site, soils irrigated with wastewater showed significant
221 highest contents of soil carbon, phosphatase activity and available phosphorous. In
222 addition, a significant decrease in pH was observed in this area compared with its
223 control site. There were no statistical differences for the rest of the parameters. In the
224 Monforte site there were statistical differences between the types of irrigation applied,
225 with mean values moderately higher for SOC, and CEC, in the soils irrigated with
226 treated wastewater. In the Biar 1 site only a moderate increase of EC was observed in
227 soils irrigated with treated wastewater with respect to the soils irrigated with fresh
228 water. A slight increase in available phosphorus was also detected. The rest of the
229 parameters suffered almost no variation due to the type of water used for irrigation.

230 All the parameters analyzed showed statistical differences because of the use of
231 wastewater, except for WHC. In this case irrigation with wastewater of lower quality
232 has produced relevant changes in almost all soil properties. Soil organic carbon content
233 has increased from 3 to 88 g kg⁻¹. EC has increased more than twice from its initial
234 values. The same behaviour was observed for the percentage of stable aggregates, and
235 all the enzymatic activities analyzed were higher in the soils irrigated with wastewater
236 in this area.

237 *3.2. Application of the soil quality indices (SQI)*

238 Residuals have been calculated and represented by different graphs for each area
239 of study. SQI 1 is showed in figure 2 and SQI 2 in figure 3. In none of the agricultural
240 soils of the study, SOCc was similar to SOCa, the residuals of two models being over
241 the limits of calibration for all of the study sites, indicating disequilibrium among soil
242 properties in all cases.

243 *3.2.1. Application of SQI 1*

244 Residuals for model 1 of all the study sites showed disequilibrium among soil
245 properties, this unbalanced situation being lower for Monforte site (MC: -0.40 and MR:
246 -0.61). The disequilibrium in soils irrigated with wastewater was moderately higher than
247 their respective controls. The highest value for SQI 1 was obtained in Biar 2 site (B2R:
248 11.14 and B2C: 2.74), the mean values of SQI 1 for the Alicante site were: -0.87 (AC)
249 and -1.49 (AR), and for the Biar 1 site were: 1.41 (BC) and 1.76 (BS).

250 *3.2.2. Application of SQI 2*

251 All soils in the study showed positive residuals for SQI 2 and a higher
252 confidence interval than established for the equilibrium. There were statistical
253 differences between treatments for each study site, soils with wastewater irrigations
254 showing higher residuals values than soils with fresh water irrigation. For this model,
255 Biar 1 showed the lowest residuals (B1C: 1.19 and B1R: 1.37) and maximum values
256 were for Alicante site (AR: 6.10 and AC: 4.78). The values of SQI 2 in the Monforte
257 site were: 3.41 (MC) and 6.00 (MR), in Biar 2 area were: 2.23 (B2C) and 4.33 (B2R).

258 **4. Discussion**

259 Some changes in the studied soil properties were found in A, M and B1 sites as a
260 consequence of the use of wastewater, (Table 2) although in general terms these
261 changes were not quite relevant in absolute values due to the good quality of treated
262 wastewater used for the irrigation in those cases (Russan et al., 2007). In soils of B1
263 area an increase of the electrical conductivity was observed as a consequence of the
264 irrigation with treated wastewater; although at the moment of sampling this value was
265 not high, it could be a risk in the long-term for soil, as it indicates an increase of the
266 saline concentration. As a consequence, electrical conductivity of water must be
267 periodically controlled to avoid undesirable effects (Morugán-Coronado et al., 2011).
268 The observed marked differences in the soils from B2 site are due to the characteristics

269 of the wastewater used, which was almost untreated and therefore rich in organic
270 compounds in this case. The long-term irrigation with this type of water caused a high
271 increase of soil organic matter content (Jueschke et al., 2008) and as a consequence
272 other properties such as AS, enzymatic activities, etc. Also in this case the value of EC
273 reached showed an important increase in soil salinity.

274 The application of the soil quality indices indicated that the use of soil for
275 agriculture caused a disturbance in its natural balance in the four different sites. Other
276 authors verify that soil tillage and fertilization caused an disequilibrium situation
277 between organic matter content and other soil properties (Karlen et al., 1994; Hussian et
278 al., 1999; Wander and Bollero, 1999; Zornoza et al., 2008).

279 Many studies have elaborated soil quality indices in agro-ecosystems using
280 different indicators (Caravaca et al., 2002; Bastida et al., 2006). These indices are
281 generally useful to classify the soils according to their degree of alteration, evaluating
282 the effects of management, the crop yield and quantifying the long-term effects of
283 different fertilisers (Glover et al., 2000; Caravaca et al., 2002; Masto et al., 2007).
284 Leirós et al. (1999) verified the usefulness of application of quality indices to
285 differentiate between different levels of degradation and the changes of soil physical,
286 chemical and biochemical properties (Burke et al., 1995).

287 In our study, the areas irrigated with wastewater (A, B1 and B2) showed higher
288 residuals values than areas irrigated with fresh water. B2R, the area with highest values
289 for the studied soil properties, was the site with maximum residuals values at both SQI
290 that it could means the alteration in this area is more severe than the other sites. Similar
291 results were found by Puglisi et al. (2006) in soils with intensive agricultural
292 exploitation, municipal and industrial wastes amendments and in soils irrigated with

293 saline waters, which showed higher residuals values than native soil with climax
294 situation.

295 The Monforte site was closer to the interval of confidence established with SQI
296 1 than the other sites of our study; these results, near the limits of calibration, could
297 indicate a slight recovery of equilibrium in soil properties, that may be attributable to a
298 better management of agricultural practices during a long-term period (more than
299 twenty years with the correct irrigation and right management). SQI 1 could be
300 appropriate for assessing severe states of degradation, like in the case of B2R site, due
301 to its susceptibility to detect changes in physical and chemical properties of soil (Filip,
302 2002; Reynolds et al., 2002).

303 SQI 2 is more sensitive than SQI 1, owing to the high sensitivity to biochemical
304 indicators (enzyme activities) (Nannipieri et al., 1990; Dick et al., 1996; Van Brugger
305 and Semenov, 2000). Biochemical properties, such as soil enzymatic activities, change
306 more quickly than physical properties. Caravaca et al. (2002) demonstrated the
307 alteration of enzymatic activities due to agricultural practices and evaluated the effects
308 of management and land use on enzymatic activities; they concluded that altered soils
309 showed higher residuals values than control soils. García-Ruíz et al. (2008) revealed
310 that intensive tillage has a tendency to delay any progress in soil quality. In contrast,
311 Bergstrom et al. (1998) focused on the effects of tillage on enzymatic activities in an
312 agricultural soil and the response was not consistent. In our case the residual values of
313 SQ2 were higher than those obtained for SQ1, pointing to its higher sensibility to soil
314 disturbances. The results confirm that SQI 1 more clearly evidences severe state of
315 degradation, while SQI 2 is more adequate to indicate initial or fast perturbations in soil.

316 The soil quality indices used in our research (Zornoza et al., 2007b) have
317 revealed the high level of disturbance in these Mediterranean agricultural soils. Tillage

318 practices are generally considered to be the major cause of soil degradation and it has
319 also been confirmed that agricultural management has caused a disruption in the natural
320 equilibrium of soils (Kieft, 1994; Gardi et al., 2002). Continuous soil tillage and
321 fertilisation have led to a disturbed situation between organic carbon and other soil
322 properties.

323 **5. Conclusions**

324 The results of our study showed that only in the case of very low waste water
325 quality and its long-term application, relevant changes in soil properties can be
326 produced.

327 The application of the soil quality indices showed that all of the soils of the
328 agricultural study sites are in a situation of disequilibrium with respect to the
329 relationships between their properties and independently of the water used for irrigation.
330 The differences of the residuals of the soil quality indices among irrigated with
331 wastewaters and their controls were low, except for the case of the site where there was
332 low quality of wastewater and a long-term application.

333 The soil quality indices seem to be useful to differentiate between degraded
334 status in agricultural soils, and can be used in monitoring and assessing the best
335 agricultural managements and water for irrigation. Nonetheless, it is necessary to
336 validate these indices in other soils and sites, and it could also be interesting to validate
337 their use for other potential causes of degradation, such as contamination, salinization,
338 compactation, management practices, etc., or how they respond to distinct practices of
339 soil recovery. This validation would also be required to determine the precise suitability
340 of each model for a concrete cause of degradation.

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349 **References**

- 350 Anderson, T.H., 2003. Microbial eco-physiological indicators to assess soil quality.
351 *Agriculture Ecosystems & Environment* 98, 285-293.
- 352 Andrews, S.S., Carroll, C.R., 2001. Designing a soil quality assessment tool for
353 sustainable agroecosystem management. *Ecological Applications* 11, 1573-1585.
- 354 Aon, M.A., Cabello, M.N., Sarena, D.E., Colaneri, A.C., Franco, M.G., Burgos, J.L.,
355 Cortassa, S., 2001. I. Spatio-temporal patterns of soil microbial and enzymatic
356 activities in an agricultural soil. *Applied Soil Ecology* 18, 239-254.
- 357 Asano, T., 1991. Planning and Implementation of Water Reuse Projects. *Water Science*
358 *& Technology* 24, 1-10.
- 359 Badiane, N.N.Y., Chotte, J.L., Pate, E., Masse, D., Rouland, C., 2001. Use of enzyme
360 activities to monitor soil quality in natural and improved fallows in semi-arid
361 tropical regions. *Applied Soil Ecology* 18, 229-238.
- 362 Bastida, F., Moreno, J.L., Hernández T., García, C., 2006. Microbiological degradation
363 index of soils in a semi-arid climate. *Soil Biology & Biochemistry* 38, 3463-
364 3473.
- 365 Bastida, F., Zsolnay, A., Hernández, T., García, C., 2008. Past, present and future of
366 soil quality indices: A biological perspective. *Geoderma* 147, 159-171.
- 367 Bergstrom, D.W., Monreal, C.M., King, D.J., 1998. Sensitivity of soil enzyme activities
368 to conservation practices. *Soil Science Society of American Journal* 62, 1286-
369 1308.
- 370 Brar, M.S., Khurana, M.P.S., Kansal, B.D., 2002. Effect of irrigation by untreated
371 sewage effluents on the micro and potentially toxic elements in soils and plants,
372 Department of Soils, Punjab Agricultural University, Ludhiana, Punjab, India.

373 Burke, I.C., Elliot, E.T., Cole, C.V., 1995. Influence of macroclimate, landscape
374 position, and management on soil organic matter in agroecosystems. *Ecological*
375 *Applications* 5, 124-131.

376 Burns, J.C., Westerman, P., King, L.D., Cummings, G.A., Ovescash, M.R., Goode, L.,
377 1985. Swine lagoon effluent applied to coastal bermudagrass: Forage yield,
378 quality and elemental removal. *Journal of Environmental Quality* 14, 9-14.

379 Caldwell, B.A., Griffiths, R.P., Sollins, P., 1999. Soil enzyme response to vegetation
380 disturbance in two lowland Costa Rican soils *Soil Biology & Biochemistry*. 31,
381 1603-1608.

382 Caravaca, F., Masciandaro, G., Ceccanti, B., 2002. Land use in relation to soil chemical
383 and biochemical properties in a semi-arid Mediterranean environment. *Soil &*
384 *Tillage Research* 68, 23-30.

385 Chapman, S.J., Campbell, C.D., Puri, G., 2003. Native woodland expansion: soil
386 chemical and microbiological indicators of change. *Soil Biology & Biochemistry*
387 35, 753-764.

388 Day, A.D., Rahman, A., Katterman, F.R.H., Jensen, V., 1974. Effects of treated
389 municipal wastewater and commercial fertilizer on growth, fiber, acid-soluble
390 nucleotides, protein, and amino acid content in wheat hay. *Journal of*
391 *Environmental Quality* 3, 17-19.

392 Dick, R.P., Breakwell, D.P., Turco, R.F., 1996. Soil enzyme activities and biodiversity
393 measurements as integrative microbiological indicators. En: Doran, J.W., Jones,
394 A.J. (Eds.), *Methods for Assessing Soil Quality*. Soil Science Society of America,
395 Special Publication 49, Madison, WI, pp 247-271.

396 Díez, J.A., 1982. Consideraciones sobre la utilización de la técnica extractiva de
397 Burriel-Hernando para la evaluación de fósforo asimilable en suelos. *Anales de*
398 *Edafología Agrobiológica* 41 (7-8), 1345-1353.

399 Emmerling, C., Udelhoven, T., 2002. Discriminating factors of the spatial variability of
400 soil quality parameters at landscape-scale. *Journal of Plant Nutrition and Soil*
401 *Science* 165, 706-712.

402 EPSAR, 2010. Purified and reused volumes of the waste water treatment plants in the
403 province of Alicante, 2008-09.

404 Filip, Z., 2002. International approach to assessing soil quality by ecologically-related
405 biological parameters. *Agriculture, Ecosystems & Environment* 88, 169-174.

406 Friedel, J.K., Langer, T., Siebe, C., Stahr, K., 2000. Effects of long-term waste water
407 irrigation on soil organic matter, soil microbial biomass and its activities in central
408 Mexico. *Biology & Fertility of Soils* 31, 414-421.

409 Forster, J.C., 1995. Soil physical analysis. In: Alef, K. and Nannipieri, P. (eds.)
410 *Methods in Applied Soil Microbiology and Biochemistry*. Academic Press Inc.
411 San Diego, CA, p. 106.

412 García-Ruíz, R., Ochoa, V., Hinojosa, M.B., Carreira, J.A., 2008. Suitability of enzyme
413 activities for the monitoring of soil quality improvement in organic agricultural
414 systems. *Soil Biology & Biochemistry* 40, 2137-2145.

415 Gardi, C., Tomaselli, M., Parisi, V., Petraglia A., Santini, C., 2002. Soil quality
416 indicators and biodiversity in northern Italian permanent grasslands. *European*
417 *Journal of Soil Biology* 38, 103-110.

418 Gee, G.W., Bauder, J.W., 1986. Particle size analysis. *Methods of soil analysis. Part 1.*
419 *Physical and mineralogical methods*. 2nd Ed. A.S.A., Madison, WI. 9, 383-441.

420 Glover, J.D., Reganold, J.P., Andrews, P.K., 2000. Systematic method for rating soil
421 quality of conventional, organic, and integrated apple orchards in Washington
422 State, *Agriculture Ecosystems & Environment* 80, 29-45.

423 Hussain, I., Olson, K.R., Wander, M.M., Karlen, D.L., 1999. Adaptation of soil quality
424 indices and application to three tillage systems in southern Illinois. *Soil & Tillage
425 Research* 50, 237-249.

426 Jueschke, E., Marschner, B., Tarchitzky, J., Chen, Y., 2008. Effects of treated
427 wastewater irrigation on the dissolved and soil organic carbon in Israeli soils.
428 *Water Science Technology* 57, 727-733.

429 Karlen, D.L., Wollenhaupt, N.C., Erbach, D.C., Berry, E.C., Swan, J.B., Eash, N.S.,
430 Jordhal, J.L., 1994. Long-term tillage effects on soil quality. *Soil & Tillage
431 Research* 32, 313-327.

432 Karlen, D.L., Mausbach, M.J., Doran, J.W., Cline, R.G., Harris, R.F., Schuman, G.E.,
433 1997. Soil quality: a concept, definition, and framework for evaluation. *Soil
434 Science Society of American Journal* 61, 4-10.

435 Kieft, T.L., 1994. Grazing and plant-canopy effects on semi-arid soil microbial biomass
436 and respiration. *Biology & Fertility of Soils* 18, 155-162.

437 Keremane, G.B., Mckay, J., 2008. Water reuse projects: The role of community social
438 infrastructure. *Water* 35, 35-39.

439 Leirós, M.C., Trasar-Cepeda, C., García-Fernández, F., Gil-Sotres, F., 1999. Defining
440 the validity of a biochemical index of soil quality. *Biology & Fertility of Soils* 30,
441 140-146.

442 Lentzsch, P., Wieland, R., Wirth, S., 2005. Application of multiple regression and
443 neural network approaches for landscape-scale assessment of soil microbial
444 biomass. *Soil Biology & Biochemistry* 37, 1577-1580.

445 Lin, C.C., Young, C.C., Lin, H.Z., Huang, S.N., 2006. The changes of dissoluble
446 organic carbon in submerged soil amended with green manures. *Research Bulletin*
447 *Tainan District Agricultural Research and Extension Station* 47, 17-30.

448 Masto, R.E., Chhonkar, P.K., Singh, D., Patra, A.K., 2007. Soil quality response to
449 long-term nutrient and crop management on a semi-arid Inceptisol. *Agriculture*
450 *Ecosystems & Environment* 18, 130-142.

451 Mekki, A., Dhouib, A., Aloui, F., Sayadi, S., 2006. Olive wastewater as an ecological
452 fertiliser. *Agronomy for Sustainable Development* 20, 61-67.

453 Mohammad, M.J., Mazahreh, N., 2003. Changes in soil fertility parameters in response
454 to irrigation of forage crops with secondary treated waste water. *Soil Science and*
455 *Plants Analysis* 34, 1281-1294.

456 Morugán-Coronado, A., García-Orenes, F., Mataix-Solera, J., Arcenegui, V., Mataix-
457 Beneyto, J., 2011. Short-term effects of treated wastewater irrigation on
458 Mediterranean calcareous soil. *Soil Tillage & Research* 112, 18-26.

459 Nannipieri, P., Ceccanti, B., Cervelli, S., Matarese, E., 1980. Extraction of phosphatase,
460 urease, protease, organic carbon and nitrogen from soil. *Soil Science Society of*
461 *America Journal* 44, 1011-1016.

462 Nannipieri, P., Grego, S., Ceccanti, B., 1990. Ecological significance of the biological
463 activity in soils. In: J.M. Bollag and G. Stotzky, Editors, *Soil Biochemistry*,
464 Marcel Dekker, New York, 293-355.

465 Pedrero, F., Kalavrouziotis, I., Alarcón, J.J., Koukoulakis, P., Asano, T., 2010. Use of
466 treated municipal wastewater in irrigated agriculture-Review of some practices in
467 Spain and Greece. *Agricultural Water Management* 97, 1233-1241.

468 Pescod, M.B., 1992. Wastewater treatment and use in agriculture. *Irrigation and*
469 *drainage*. Paper No. 47. FAO, pp 118.

470 Puglisi, E., Nicelli, M., Capri, E., Trevisan, M., Del Re, A.A.M., 2005. A soil alteration
471 index based on phospholipid fatty acids. *Chemosphere* 61, 1548-1557.

472 Puglisi, E., Del Re, A.A.M., Rao, M.A., Gianfreda, L., 2006. Development and
473 validation of numerical indices integrating enzyme activities of soils, *Soil Biology*
474 *& Biochemistry* 38, 1673-1681.

475 Rattan, R.K., Datta, S.P., Chhokar, P.K., Suribabu, K., Singh, A.K., 2005. Long-term
476 impact of irrigation with sewage effluents on heavy metal content in soils, crops
477 and groundwater-a case study. *Agriculture, Ecosystems & Environment* 109, 310-
478 322.

479 Reynolds, W.D., Bowman, B.T., Drury, C.F., Tan, C.S., Lu, X., 2002. Indicators of
480 good soil physical quality: density and storage parameters. *Geoderma* 110, 131-
481 146.

482 Roig, A., Romero, M., Lax, A., Fernández, F.G., 1980. Estudio comparativo de métodos
483 de determinación de capacidad de cambio catiónica en suelos calizos. *Anales de*
484 *Edafología y Agrobiología* 39, 2021-2032.

485 Roldán, A., García-Orenes, F., Lax, A., 1994. An incubation experiment to determinate
486 factors involving aggregation changes in an arid soil receiving urban refuse. *Soil*
487 *Biology & Biochemistry* 26, 1699-1707.

488 Rosabal, A., Morillo, E., Undabeytia, T., Maqueda, C., Justo, A., Herencia, J.F., 2007.
489 Long-term impacts of wastewater irrigation on Cuban soils. *Soil Science Society*
490 *of America Journal* 71, 1292-1298.

491 Ruf, A., Beck, L., Dreher, P., Hund-Rinke, K., Römbke, J., Spelda, J., 2003. A
492 biological classification concept for the assessment of soil quality: "biological soil
493 classification scheme" (BBSK). *Agriculture, Ecosystems & Environment* 98, 263-
494 271.

495 Russan, M.J.M., Hinnawi, S., Rousan, L., 2007. Long-term effect of wastewater
496 irrigation of forage crops on soil and plant quality parameters. *Desalination* 215,
497 143-152.

498 Salamanca, E.F., Raubuch, M., Joergensen, R.G., 2002. Relationships between soil
499 microbial indices in secondary tropical forest soils. *Applied Soil Ecology* 21, 211-
500 219.

501 Sparling, G.P., Schipper, L.A., Bettjeman, W., Hill, R., 2004. Soil quality monitoring in
502 New Zealand: practical lessons from a 6-year trial. *Agriculture, Ecosystems &*
503 *Environment* 104, 523-534.

504 Tabatabai, M.A., Bremner, J.M., 1969. Use of *p*-nitrophenyl phosphate in assay of soil
505 phosphatase activity. *Soil Biology & Biochemistry* 1, 301-307.

506 Tabatabai, M.A., 1982. Soil enzymes. In: Page, A.L., Miller, R.H., Keeney, D.R. (Eds.),
507 *Methods of Soil Analysis, Part 2*, 2nd ed. Agronomical Monograph No. 9,
508 American Society of Agronomy and Soil Science of America, Madison, WI, pp.
509 501-538.

510 Trasar-Cepeda, C., Leirós, C., Gil-Sotres, F., Seoane, S., 1998. Towards a biochemical
511 quality index for soils: An expression relating several biological and biochemical
512 properties. *Biology & Fertility of Soils* 26, 100-106.

513 Van Bruggen, A.H.C., Semenov, A.M., 2000. In search of biological indicators for soil
514 health and disease suppression. *Applied Soil Ecology* 15, 13-24.

515 Walkley, A., Black, I.A., 1934. An examination of the Degtjareff method for
516 determining soil organic matter and a proposed modification of the chromic acid
517 titration method. *Soil Science* 37, 29-38.

518 Wander, M.M., Bollero, G.A., 1999. Soil Quality Assessment of Tillage Impacts in
519 Illinois. *Soil Science Society of America Journal* 63, 961-971.

520 Wick, B., Kühne, R.F., Vielhauer, K., Vlek, P.L.G., 2002. Temporal variability of
521 selected soil microbiological and biochemical indicators under different soil
522 quality conditions in south-western Nigeria. *Biology & Fertility of Soils* 35, 155-
523 167.

524 Weber, B., Avnimelech, Y., Juanico, M., 1996. Salt enrichment of municipal sewage:
525 New prevention approaches in Israel. *Environment Management* 20, 487-495.

526 Zornoza, R., Mataix-Solera, J., Guerrero, C., Arcenegui, V., García-Orenes, F., Mataix-
527 Beneyto, J., Morugán, A., 2007a. Evaluation of soil quality using multiple lineal
528 regression based on physical, chemical and biochemical properties, *Science of the*
529 *Total Environment* 378, 233-237.

530 Zornoza, R., Mataix-Solera, J., Guerrero, C., Arcenegui, V., Mayoral, A.M., Morales, J.
531 Mataix-Beneyto, J., 2007b. Soil properties under natural forest in the Alicante
532 Province of Spain. *Geoderma* 142, 334-341.

533 Zornoza, R., Mataix-Solera, J., Guerrero, C., Arcenegui, V., Mataix-Beneyto, J.,
534 Gómez, I., 2008. Validating the effectiveness and sensitivity of two soil quality
535 indices based on natural forest soils under Mediterranean conditions. *Soil Biology*
536 *& Biochemistry* 40, 2079-2087.

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538 **Table 1.** Study sites, irrigation methods, climatic parameters and soil characteristics.

Site ^a	Water type used ^b	Period of irrigation (years)	Irrigation method	Tm ^c (°C)	Pm ^c (mm)	Soil Type (SSS, 2010)	Texture ^d (% sand, silt clay)	OM ^e (%)	pH	CaCO ₃ (%)
AC	FW	40	Drip			Xerorthent	Silty loam (2,56,42)	3.8	8.3	53
AR	WW	40	Drip	17.9	301	Xerorthent	Silty clay loam (7,77,16)	5.0	7.9	54
MC	FW	20	Drip			Xerorthent	Clay loam (56,22,22)	3.2	8.3	46
MR	WW	20	Drip	18.3	335	Xerorthent	Clay loam (56,20,24)	4.5	8.1	48
B1C	FW	2	Flooding				Silty clay loam (30,45,25)	2.0	8.6	42
B1R	WW	2	Flooding	14.5	486	Xerofluvent	Clay loam (26,45,29)	2.0	8.9	43
B2C	-	-	-				Sandy clay loam (74,8,18)	1.0	8.7	25
B2R	WW	20	Flooding	14.5	486	Xerofluvent	Sandy clay loam (60,18,22)	8.0	7.5	34

539 ^a A: Alicante; M: Monforte; B: Biar; C: Control plots without use of wastewater; R: plots with wastewater irrigation

540 ^b FW: fresh water; WW: wastewater

541 ^c Mean annual temperature (Tm) and mean annual precipitation (Pm).

542 ^d Sand: 2-0.02 mm; silt: 0.02-0.002 mm; clay: <0.002 mm.

543 ^e OM: organic matter.

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546 **Table 2.** Mean values of main properties of irrigation wastewaters used in the study.

Parameter	B1R	B2R	AR	MR
EC ($\mu\text{S cm}^{-1}$)	1915	4000	3088	1856
BOD ₅ ($\text{mgO}_2 \text{l}^{-1}$)	13.5	337	13	19
COD ($\text{mgO}_2 \text{l}^{-1}$)	59.6	819	52	74
SS (mg l^{-1})	18.5	207	22	19

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EC: Electrical conductivity; BOD₅: biological oxygen demand; COD: Chemical Oxygen Demand; SS: suspended solids.

551 **Table 3.** Mean values \pm standard deviation and t-student values of the soil properties for each site.

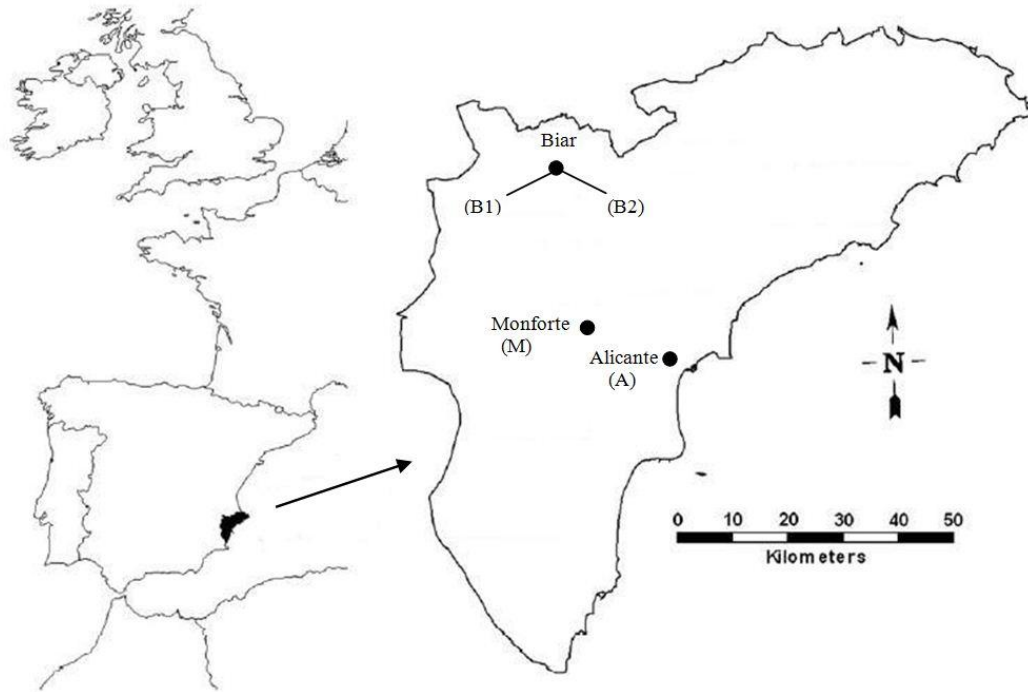
Site ^a	SOC	pH	EC	CEC	phosphorus	WHC	AS	Phosphatase	Urease	B-glucosidase
	g kg ⁻¹		$\mu\text{S cm}^{-1}$	cmol ⁺ kg ⁻¹	mg kg ⁻¹	%	%	$\mu\text{mol PNP g}^{-1} \text{h}^{-1}$	$\mu\text{mol NH}_4^+ \text{g}^{-1} \text{h}^{-1}$	$\mu\text{mol PNP g}^{-1} \text{h}^{-1}$
AC	22 \pm 4	8.3 \pm 0.1	412 \pm 28	10.2 \pm 0.9	163.4 \pm 29.3	52 \pm 3	40 \pm 16	1.52 \pm 0.46	7.54 \pm 1.63	1.96 \pm 0.41
AR	30 \pm 2	7.9 \pm 0.1	496 \pm 83	9.8 \pm 1.0	213.8 \pm 27.4	52 \pm 3	58 \pm 16	2.29 \pm 0.37	9.29 \pm 1.78	2.18 \pm 0.19
t	-4.5***	6.8***	-2.4*	n.s.	-3.1*	n.s.	n.s.	-3.3*	n.s.	n.s.
MC	19 \pm 4	8.3 \pm 0.1	339 \pm 61	6.1 \pm 0.6	109.8 \pm 19.8	50 \pm 2	45 \pm 5	1.20 \pm 0.32	4.83 \pm 0.97	1.58 \pm 0.46
MR	26 \pm 2	8.1 \pm 0.1	478 \pm 97	7.6 \pm 1.6	133.4 \pm 9.9	55 \pm 8	39 \pm 10	1.92 \pm 0.81	4.89 \pm 1.02	1.12 \pm 0.44
t	9.7***	-3.1*	n.s.	7.6***	11.5***	4.9**	7.7***	8.3***	11.3***	7.7**
B1C	12 \pm 1	8.6 \pm 0.1	186 \pm 24	5.5 \pm 0.3	14.2 \pm 1.5	50 \pm 3	49 \pm 5	0.60 \pm 0.09	1.60 \pm 0.44	0.49 \pm 0.08
B1R	12 \pm 1	8.9 \pm 0.1	359 \pm 33	5.9 \pm 0.8	19.7 \pm 3.9	49 \pm 6	42 \pm 8	0.63 \pm 0.09	1.50 \pm 0.35	0.54 \pm 0.13
t	n.s.	7.1***	10.4***	n.s.	3.2*	n.s.	n.s.	n.s.	n.s.	n.s.
B2C	3 \pm 1	8.9 \pm 0.1	76 \pm 9	1.0 \pm 0.2	19.3 \pm 1.9	27 \pm 2	36 \pm 13	0.28 \pm 0.08	5.93 \pm 3.79	0.41 \pm 0.16
B2R	88 \pm 16	7.8 \pm 0.1	160 \pm 20	18.0 \pm 3.7	234.9 \pm 34.0	25 \pm 7	85 \pm 1	3.31 \pm 0.81	34.83 \pm 6.79	2.07 \pm 0.15
t	13.2***	33.7***	-9.2***	-11.3***	-15.5***	n.s.	-8.9***	64.8***	-9.1***	-18.9***

552 ^a AC: Alicante Control; AR: Alicante with wastewater irrigation; MC: Monforte Control; MR: Monforte with wastewater
553 irrigation; B1C: Biar 1 Control; B1R: Biar 1 with wastewater irrigation; B2C: Biar 2 control; B2R: Biar 2 with wastewater
554 irrigation. SOC: soil organic carbon; EC: electrical conductivity; CEC; cation exchange capacity; available phosphorus; WHC:
555 water holding capacity; AS: aggregate stability. PNP: *p*-nitrophenol. Different letters indicate significant differences. (Significant at:
556 P<0.05 = *, P<0.01 = **, P<0.001 = ***, n.s. = not significant = P>0.05)

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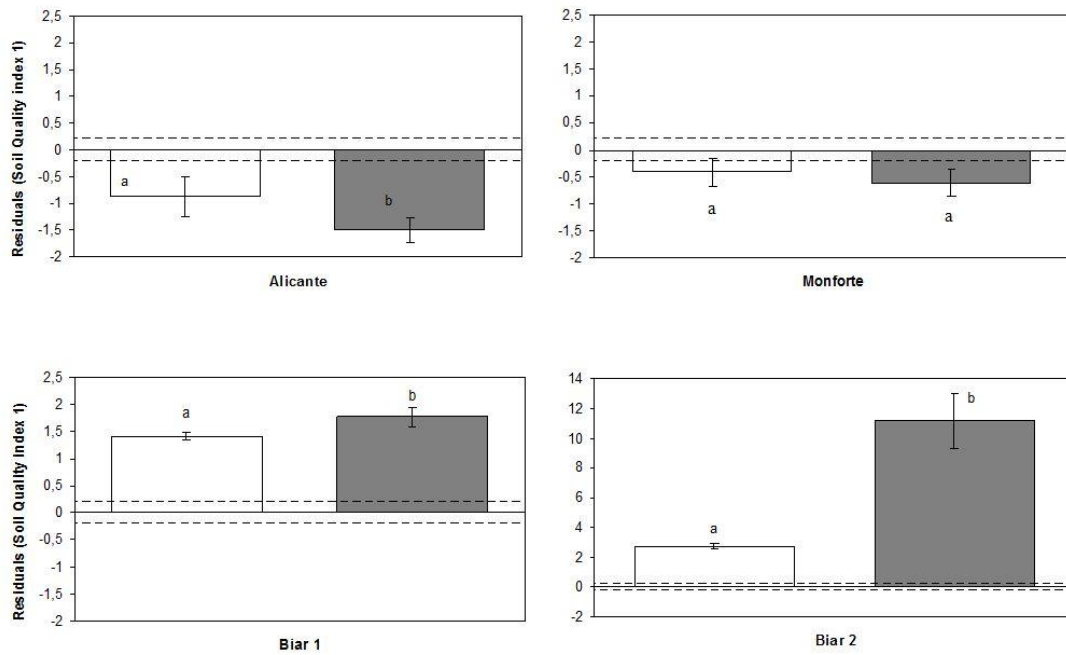
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562 **Figure 1.** Location of the study sites.

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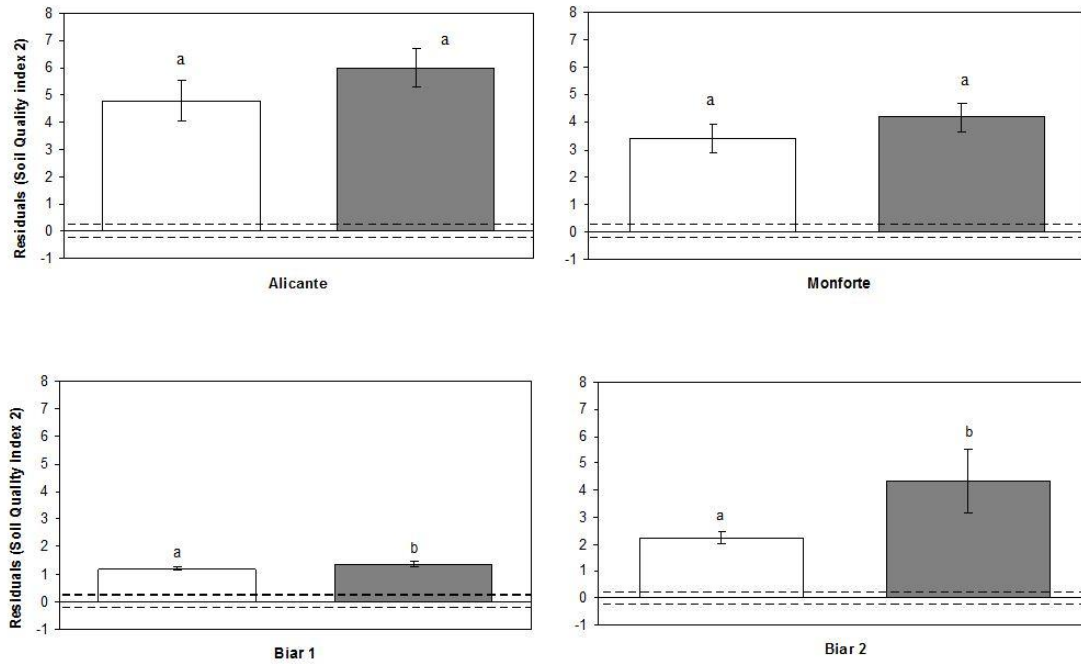


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565 **Figure 2.** Soil quality index 1 for each of the study sites. Dash lines limit 95%
 566 confidence interval of calibration (-0.21, +0.21). Controls are indicated with white color
 567 bars and irrigated with wastewaters with dark grey color. Different letters indicate
 568 significant differences between means at ($P < 0.05$) after T-Student test. Error bars
 569 denote standard deviation.

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573 **Figure 3.** Soil quality index 2 for each of the study sites. Dash lines limit 95%
 574 confidence interval of calibration (-0.23, +0.23). Controls are indicated with white color
 575 bars and irrigated with wastewaters with dark grey color. Different letters indicate
 576 significant differences between means at ($P < 0.05$) after T-Student test. Error bars
 577 denote standard deviation.