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Assessment of vulnerable freshwater ecosystems and various aquatic effluents by means of ecotoxicological assays

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ABSTRACT

A battery of ecotoxicological assays of different trophic chain levels has been performed for water and sediment samples of two Greek vulnerable freshwater ecosystems (Karla Lake in Thessaly Region and Koronia Lake in Central Macedonia Region, respectively). These lakes, despite remediation programs, are still characterized by varied water quality. Furthermore, the quality of aquatic reservoirs aimed for human consumption as well as this of treated and untreated wastewater samples was also assessed through these bioassays. The outcome of the present research shows that condition of the studied lake ecosystems is still poor and intervention is needed for rectification of their continuous decline. Samples from treated municipal wastewater from the same regions were of medium to satisfactory quality, as measured by ecotoxicological assays. Despite causative relationships between burdened physicochemical parameters and increased toxicity, this toxicity could not always be attributed to these parameters. Results of bioassays in combination with standard physicochemical measurements of water samples may aid the integrated assessment of environmental risk arising from presence or release of these water samples in the ecosystem.

Keywords: Water quality; Bioassays; Karla Lake; Koronia Lake; *D. magna*; *V. fischeri*; *P. subcapitata*

1. Introduction

Water quality may be influenced by a wide range of natural factors (biological, geological, hydrological, meteorological, and topographical). These factors may affect substantially water suitability and may vary seasonally according to differences in weather conditions, run-off volumes, and water levels. An equally important parameter on water quality is the human effect. The human pressure may be demonstrated as hydrological influence via flow diversion, water abstraction, wetland drainage, or dam construction. However, it is mostly substantiated as quality decline due to pollution, discharge of sewage, agricultural, industrial, and urban wastewater, run-off of fertilizers and agrochemicals,

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and leaching of by-products of various industrial and municipal processes are omnipresent factors around the world [1]. Nowadays, water pollution is on the rise globally despite focal improvements in some regions. Established problems like microbial contamination of potable waters or enhancement of eutrophication are further exacerbated due to increasing wastewater effluents associated with an increasing population over the next three decades, continuation of untreated discharge in rivers, lakes, and coastal areas in developing countries and reallocation of industries from high-income countries to emerging-market economies [2]. Basins of relatively still water such as natural or artificial lakes are currently at high risk, facing a number of challenges including urban development and sprawl, change of land uses, and intensification of agriculture. Furthermore, global climate alterations and continuous expansion of invasive species [3,4 respectively] may act synergistically, worsening the effects of anthropogenic pollution.

Quality of water and wastewater samples may be quantified via different kinds of analyses:

- physicochemical analyses which determine various physical properties of the water sample including color and odor, electrical conductivity, pH, temperature, total suspended solids, and others;
- chemical analyses which detect and quantify a number of possible pollutants found in the water sample via analytical techniques. The list of the pollutants examined each time is relative to the desirable use of the water sample; for drinking water the pollutants that have to be looked for are numerous and the detectable limits are strict [5]. Reclaimed wastewater for irrigation is also characterized by stringent criteria [6] which are further modified on the basis of the irrigated product (crops commercially/not commercially processed, landscape irrigation, non-food crops, and others);
- microbiological analyses which define and quantify groups of micro-organisms, characteristic of existence of hazard for public health [5]; and
- finally, ecotoxicological analyses (also known as bioassays) are also performed on water samples utilizing standardized protocols on living organisms of various trophic levels [7]. These analyses observe and quantify the toxicity on the examined species, which is related to the pollution burden of the water sample.

Bioassays may prove extremely useful as complementary or even as precedent to chemical analyses since it is well known that: (1) detection and quantification of pollutants in small quantities are often cumbersome and expensive; (2) combined action of these pollutants in aquatic organisms cannot be highlighted through chemical analyses; and (3) bioaccumulation, bioavailability, and transport through food chains are also not quantified. Conducting ecotoxicological assays in parallel to chemical analyses, answers some of these questions, since these tests assess the effect of polluted aquatic specimens on survival, growth, and other basic functions on organisms of different trophic levels. Results are then compared with organisms not exposed to the polluted aquatic samples or to set levels of toxicity, so that useful deductions on the toxic potential of the examined water samples can be derived.

Bioassays have been proven valuable for ranking and monitoring freshwater reservoirs around the world; for example, rivers Shibuta and Tama in Japan were tested regarding their toxicity to *Pseudokirchneriella subcapitata* and they were subsequently classified on an "index biosafety rank," [8] as well as Lake Biwa which exhibited high toxicity on *P. subcapitata* during an agrochemical effluent peak [9]. Furthermore, specimens from polluted river outlets in Poland were tested on *Daphnia magna*, *P. subcapitata*, *Vibrio fischeri*, and other similar organisms [10] enabling a realistic acute hazard classification of these waters. Finally, sediment extracts from a large artificial lake in Portugal showed a gradient of acute and chronic toxicity in the species used especially in the invertebrate *Heterocypris incongruens* [11].

Taking all these into consideration, two vulnerable freshwater ecosystems in dire need of remediation, Karla Lake in Thessaly Prefecture and Koronia Lake in Central Macedonia Prefecture, have been chosen for the present study. Karla Lake is supposed to undergo extensive remediation procedures [12] since, it is a partially reconstructed lake utilizing river Pinios sources. Unfortunately, saline soils of the former natural lake bed affect the reservoir due to leaching in the drainage system during the wet season. Furthermore, the dual action of the established drainage–irrigation system facilitates a continuous recycling of salts and other pollutants mainly agrochemicals used in Thessaly plain [13]. Koronia Lake in Central Macedonia Prefecture is also affected by numerous unfavorable parameters despite been characterized as a site of international importance for migrating and indigenous birds [14]. Water quality is declining due to negative water balance and to input of substantial amounts of pollutants from point and diffuse sources including small-scale dairy, textile, agricultural, and metal finishing industries.

In the present research, toxicity assessment of lake water or sediment on selected organisms, the bacterium *V. fischeri*, the crustacean *D. magna*, and the algae *P. subcapitata*, has been performed according to relevant accredited protocols, in parallel to requisite physicochemical tests. Results depict the current status of water quality of the lakes. Similar results were drawn for other aquatic reservoirs also in the examined areas of Northern Greece: treated and untreated water samples or groundwater samples aimed for human consumption were examined in relation to their ecotoxicity potential. The central aims of the project were the following:

- to monitor the current status of two vulnerable ecosystems under lengthy remediation procedures which, despite significant efforts, show indications of continuous deterioration;
- to assess the quality of groundwater and reclaimed wastewater of the wider region of the aforementioned Prefectures; and
- to correlate and combine performances based on toxicity testing outcomes and on physicochemical parameters.

The results give a comprehensive snapshot of the freshwater quality of a mainland Mediterranean region with overall water deficit and serve as part of an expanding spatiotemporal database for these regions.

2. Materials and methods

2.1. Sample collection and maintenance

All samples were collected and stored according to [15]. Samples were collected in plastic containers previously cleaned by washing with tap water and later soaked in 10% HNO_3 for 24 h and finally rinsed with deionized water prior to usage. During sampling, sample bottles were rinsed with sampled water three times and then filled to the brim. In the case of sediment, where water was scarce, bottle was filled with the collected solid and sealed. The samples were labeled, transported to the laboratory, and stored in the refrigerator at about 4°C, prior to analysis. Samples were collected in the period September and November 2012. Treated wastewater samples were collected from the municipal wastewater treatment plant (MWWTP) of Lagadas (Central Macedonia Region) and treated/untreated samples from the MWWTP of Tirnavos (Thessaly Region) in autumn 2012. Leachates from sediment of Lake Koronia (Central Macedonia Region), water samples from Lake Karla (Thessaly Region), and groundwater samples (boreholes) from different regions in Thessaly were also collected in autumn 2012 (Fig. 1).

2.2. Leachate extraction

Leachates of lake sediment were extracted according to the EN 12457-2 method [16]. In brief, the leaching test was performed at a ratio of liquid to solid sample $L/S = 10$ L/kg. A mixture of 90 g solid sample and deionized water was combined in 1 L polyethylene bottles. The latter were then agitated for 24 ± 0.5 h on a rotary agitator (Velp, Italy) equipped with an adjustable rotation device set at 10 rpm. The eluates were then collected by filtration through a 0.45 μm membrane filter. Conductivity and pH of the eluates were immediately recorded and the samples were maintained as described previously.

2.3. Physicochemical analyses

All tests were performed according to accredited methods described in [15]. In brief, COD was calculated according to 5220 B Open Reflux Method. BOD was calculated according to 5210 Biochemical Oxygen Demand Method. Nitrate levels were calculated according to 4500-NO_3^- Method and nitrite levels according to 354.1 Method. Total Organic Carbon was measured in a Shimadzu TOC-5000A Analyzer. pH was measured with a Basic 20 pH-meter (Crison, Spain), conductivity with Cond 720 (WTW, Germany), and dissolved oxygen with Oxi 315i SET (WTW, Germany).

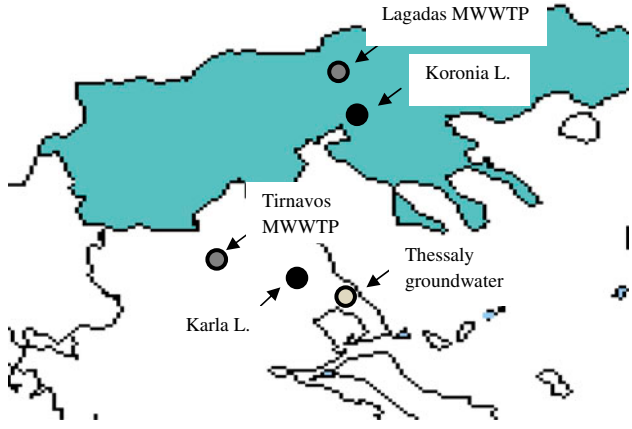

Fig. 1. Map of Northern Greece (Thessaly and Macedonia region).

2.4. V. fischeri bioluminescence test

The leachates or water samples collected were evaluated with the Microtox assay using Microtox Analyzer 500. The experimental procedure has been adapted from the official standards of several countries [France (standard AFNOR T90-320-1991), Germany (standard DIN 38412-1990)] upon exposure of the test organisms to a toxic sample and this reduction is directly related to the relative toxicity of the sample. Solution of 22% NaCl in purified water was used for normalization of salinity of all samples and controls at final concentration of 2%. The bioluminescence inhibition was calculated after 5, 15, and 30 min of incubation. The effective concentration for 50% inhibition of luminescence (EC50) after 5, 15, or 30 min incubation was calculated, where possible, with data reduction software (Microtox Omni). Purified water containing 2% NaCl was used as non-toxic control.

2.5. P. subcapitata growth inhibition test

The *P. subcapitata* test was performed according to the Standard Operational Procedure for the Algaltoxkit F TM which follows [17]. After de-immobilization of the algae from the beads, the algae were centrifuged twice (3,000 rpm, Chetti, Rotofix 32A) and resuspended in growth diluent (Microbiotests Inc.) to a final density of 10^6 algal cells/100 mL. This concentration was calculated on an algal optic density scale at 670 nm (SECOMAM Anthelie Advanced). 1 mL of algal suspension was used in each non-diluted sample and control. Test plates were incubated at 24˚C in uniform illumination of 8,000 lux, for 24, 48, and 72 h, with daily determination of algal growth with the aid of a UV spectrophotometer (SECOMAM Anthelie Advanced).

Growth inhibition was calculated according to the formula:

$$I_{A_1} = \frac{A_e - A_1}{A_e} \times 100$$

where I_{A_1} is the inhibition (%); A_1 is the average area under the growth curve for the sample; A_e is the average area under the growth curve for the sample.

2.6. D. magna immobilization test

The test procedure followed the Standard Operational Procedure of the Daphtoxkit F magna microbiotest (1996), which is in accordance with the OECD and ISO test protocols for the acute *D. magna* toxicity tests [18,19]. In brief, dormant *Daphnia* eggs were developed in 3–4 d into neonates in a reconstituted hatching medium. Each sample as well as the control was assayed in four replicates in (6 × 5) multiwell plates. Five neonates were brought into each test well containing 5 mL of test medium or sample. On completion of the transfers, each multiwell plate was sealed with a strip of parafilm and covered with its lid. Then plates were placed in an incubator set at 20˚C in darkness for 24 h. For scoring of the results, the wells of each row were checked and the number of dead or immobilized neonates was recorded vs. that of the actively swimming test organisms in each well. The test was considered valid if the mean number of dead plus immobile organisms in the control wells did not exceed 10%. Since, no EC50 values were reached, the % of immobilization was calculated from the number of immobilized neonates in the samples in relation to the immobilized neonates in the control groups.

3. Results

3.1. Physicochemical analyses

Table 1 presents the results of physicochemical parameters (mean values of the measurements carried out on different sites during 2012). Five wastewater samples (effluents) from Lagadas MWWTP were collected during August–November 2012 (one sample per month; two samples in November). Two wastewater samples were collected during October–November 2012 (one sample per month, both untreated and treated effluents) from Tirnavos MWWTP. Four sediments were collected from Lake Koronia along a distance of 1 km from the outer to the inner part of the dry Lake, in September 2012. Two (surface) samples were collected from Lake Karla in September and October 2012. A total of six samples of groundwater were collected from three different regions of Magnesia Prefecture during two sampling courses in October 2012. All physicochemical parameters were analyzed with no replicate, except TOC where each sample was analyzed in triplicate.

3.2. Ecotoxicological analyses

The toxic effect on a number of organisms of different trophic levels was examined for the water and wastewater samples collected in autumn 2012. The results are summarized in Figs. 2 and 3. It can be shown that Lagadas effluent was non-toxic to all trophic levels representatives: bacteria, algae, and invertebrates. Actually, Lagadas effluents facilitated the growth of both *P. subcapitata* and *V. fischeri*, a fact that

Table 1
Physicochemical parameters measured on different sites during 2012

Parameter	Lagadas MWWTP (*effluent*)	Tirnavos MWWTP (*influent*)	Tirnavos MWWTP (*effluent*)	Lake Koronia (*leachates*)	Lake Karla (*surface*)	Ground water (*magnesia prefecture*)
pH	8.08	8.09	7.55	8.78	7.73	8.5
Conductivity (mS/cm)	0.97	1.04	1.16	4.07	3.85	0.28
TSS (mg/L)	–	187	<2	–	–	–
Nitrates (NO_3^-) (mg/L)	37.40	–	7.13	–	19.32	1.29
Nitrites (NO_2^-) (mg/L)	0.08	–	0.11	–	<0.20	<0.20
BOD (mg/L)	<5	585	<5	–	–	–
COD (mg/L)	20	850	23	–	–	–
TOC (mg/L)	–	–	–	21.4	20.3	0.8

merits further attention. Tirnavos effluent was slightly toxic to *V. fischeri* but almost non-toxic to *Daphnia*. The untreated effluents of Tirnavos were extremely toxic to *Daphnia* and they also produced detrimental effects on *V. fischeri* and *P. subcapitata*.

The effect of water samples was variable and it was dependent on the source of the sample as well as on the organism tested. Koronia Lake leachates produced slight effects on all three organisms tested. The results on *V. fischeri* were characterized by significant intravariability related to the sediment batch tested. Karla Lake water samples also produced slight effects on *D. magna* and *V. fischeri* while for *P. subcapitata* an increase in biomass of more than 10% was noted. Surface water samples were characterized by minimal effects on the tested organisms. Slight increases were noted for *V. fischeri* and *P. subcapitata* which, however, were below the trigger value of 10%. The toxicity to *D. magna* was considered negligible.

4. Discussion

Various limits and considerations are currently globally in force regarding suitability of treated wastewater for irrigation of crops and other agricultural, municipal, or industrial purposes [e.g. 6,20,21]. Strict limitations also exist for wastewater reuse as a potable source and for effluent discharge in aquatic reservoirs. However, as mentioned in introduction, bioassays are the only means of detecting and quantifying the biological effects of wastewater effluents or of water samples toward living organisms and their ecosystems. Therefore, both analytical and ecotoxicological determinations have to be applied together for better environmental characterization of water quality. The implementation of bioassays has been adopted by a number of European countries (France, Denmark) which currently advocate their use for the risk evaluation of effluents [22]. Greek legislation is also adopting the ecotoxicological approach; The Joint Ministerial Decree 145116/2011 regarding "the definition of measures, terms and processes regarding the reclamation of wastewater treatment" dictates evaluation of reclaimed wastewater by means of the *Daphnia* immobilization test [23].

Candidate organisms for bioassays have been chosen on the basis of their representativeness of all trophic levels and their sensitivity to various pollutants. The organisms used in the present study are some of the most reliable ones; *D. magna* is highly sensitive to toxic substances, it presents short generation time, it acclimatizes easily in laboratory conditions, and the results can be measured in a relatively short period [24,25]. Algae are primary producers in the aquatic ecosystem and they are sensitive to a wide array of both inorganic and organic chemicals. Regarding the selected species *P. subcapitata*, this was ranked as the most sensitive and its sensitivity was always higher than the average sensitivity of all tested species to each compound, when three organic and three inorganic toxicants were examined [26]. Furthermore, in a comprehensive study of 27 pesticides, this species was generally (but not consistently) the most sensitive out of the four algal species used [27]. Finally, the bioluminescent bacterium *V. fischeri* has been extensively used for rapid, first tier screening of toxic chemicals,

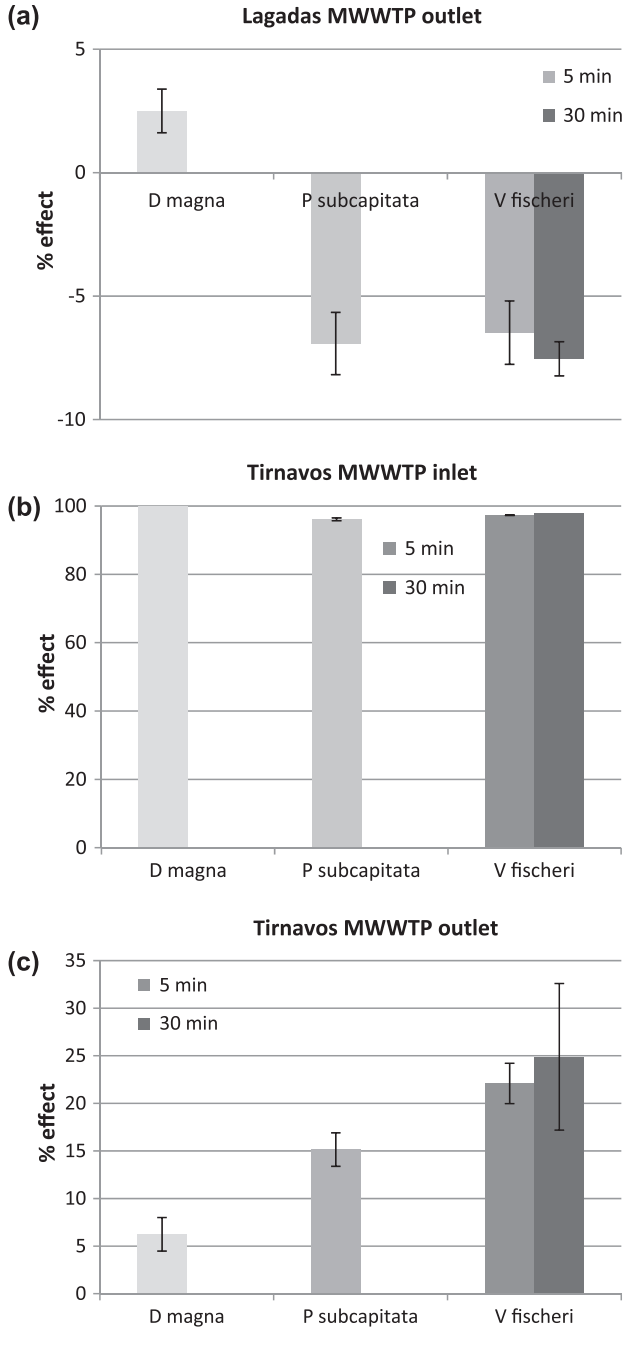

Fig. 2. Results from bioassays on MWWTP samples. (A): Lagadas outlet; (B): Tirnavos inlet; and (C): Tirnavos outlet.

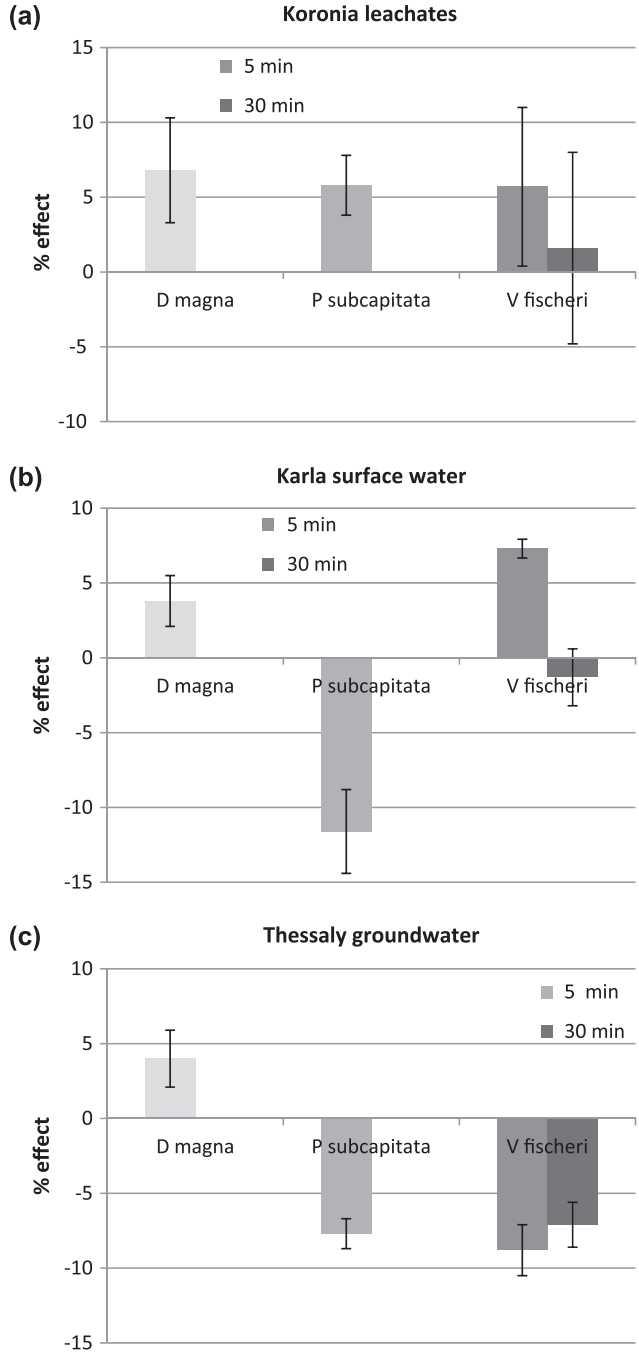

Fig. 3. Results from bioassays on surface water samples. (A): Koronia Lake leachate; (B): Karla Lake; and (C): Thessaly groundwater boreholes.

with proven sensitivity, and comparable with obligatory test species (fish, *Daphnia*, algae) [28]. A battery of bioassays of different trophic levels is strongly suggested for assessment purposes and the best compromise between labor or financial restrictions and extent of bioassays should always be sought.

As seen from the present results, untreated wastewater samples (from Tirnavos MWWTP) exhibited high toxicity in all groups of organisms. Especially for *Daphnia*, 100% mortality was noted whereas *V. fischeri* was sturdier than the cladoceran. Extrapolation in real life situations shows that this action poses an

unacceptable risk to aquatic ecosystems when municipal or industrial wastewater is directed toward aquatic reservoirs. This has been dramatically shown among others, in a study on untreated tannery effluents [29], textile industry effluents [30], and municipal effluents [31]. Despite significant amelioration of the Greek sewage system in the last decades, a number of oversights are still detected, probably due to lack of proper knowledge and maintenance. Thus, the possibility of similar occurrences (disposal of improperly treated wastewater) is not insignificant.

Previous studies on the treated effluent of the same MWWTP (Tirnavos) in the autumn of 2009 had revealed a more precarious situation with high *D. magna* mortalities [22]. It was then postulated that the effect was due to non-biodegradable pollutants which contributed to high COD values (approximately 60 mg/L in autumn 2009). This argumentation is in line with the present findings where, for the same treatment unit, lower COD values (22.5 mg/L) were accompanied by negligible toxicity toward *D. magna*. There was still slight toxicity present for *P. subcapitata* for the Tirnavos outlet as well as higher toxicity for *V. fischeri*, with outlet sample Number 1 exhibiting inhibition of 30.4% (±5.8%). *V. fischeri* has been proven to be the most sensitive organism for wastewater testing according to some researchers [32] and it was considered one of the three bioassays of high distinguishing power for a Lithuanian MWWTP [33]. On the contrary, other studies corroborate the higher sensitivity of *D. magna* and *P. subcapitata* for effluent evaluation [34]. What is worth noting is that despite Tirnavos influent being less toxic for *V. fischeri* than for *Daphnia* (where mortality was 100% with no variability), the treated effluent was still slightly toxic for *V. fischeri* whereas it was non-toxic for *Daphnia*, suggesting that the initial toxicities noted in the two examined organisms should be attributed to different pollutant groups. Different advanced wastewater treatment processes are diverse in terms of their efficiency on pollutant removal. Furthermore, it has been noted that tertiary treatment may increase undesired effects such as endocrine disruption potential *in vitro* [35]. The sole post-secondary treatment applied in Tirnavos MWWTP (sand filtration in conjunction with UV irradiation) is apparently very effective for eliminating the pollutants and the conditions eliciting toxicity in invertebrates.

Lagadas-treated effluents produced a small increase of bioluminescence of *V. fischeri*. This may be attributed to the phenomenon of hormesis—a favorable biological response to low exposures to toxicants and other stressors, which has also been previously seen for pollutants on *V. fischeri* [36]. However, we generally speak of hormesis when the observed growth is at least 10% higher than the control response [37], which was not the case here. A similar response was noted for the algae *P. subcapitata* where a small but significant increase in growth was noted. This may be attributed to the micronutrients (nitrates and phosphates) commonly found in polluted waters. Nitrate analysis in the treated wastewater revealed values ranging from 33 to 42 mg/L. These nutrients, therefore, may have aided the growth of algae and/or even masked any toxic effect caused by concomitant pollutants in wastewater. A similar response on aquatic plants has been noted for undiluted-treated effluents where the dry weight of *Lemna minor* significantly increased in relation to control [31]. The authors suggested that the primary contaminants in the effluents were nutrients and therefore, these effluents may perturb receiving water through enrichment with organic burden. The low toxicity of the treated wastewater toward *D. magna* corroborates the opinion that the treated samples of Lagadas contained low amounts of toxicants, at least for the organisms tested. Therefore, the assessment of the two MWWTP effluents revealed different potential risks; in the case of Tirnavos, toxicity to certain trophic level representatives was still present whereas in the case of Lagadas, nitrate burden may aid eutrophication of the receiving water.

Regarding possible potable sources, groundwater samples from bore holes of Koukourava, Portaria, and Alli Meria showed low toxicity for all samples on all sets of organisms with maximum values within the trigger of 10%. Furthermore, conductivity was acceptable (0.28 mS/cm) and nitrate levels were below <2 mg/L, indicating appropriate drinking quality within the limits set by 98/83/EC.

Regarding samples from the vulnerable ecosystem of Koronia, it was not feasible to obtain actual water samples. The lake and the areas around it are protected by the RAMSAR convention and have been proposed to be included in the protected areas of NATURA 2000. However, water quality of the lake has been consistently deteriorating due to negative water balance and huge amounts of pollutants received from point and diffuse sources. As noted by researchers in 2002, the lake has a maximum depth <1 m or less [38,39]. Nowadays, even after a restoration program, the situation remains precarious. Within 30 years, the lake has lost a third of its surface area and its depth has decreased from 5 to 1 m or less. In summer 2009, the lake could be walked across. In the present research, since water was scarce, sediment was collected instead, and it was subjected to the European Standard Leaching Test EN 12457-2 which has previously been used for manipulation of other solid materials [40]. It has to

be noted that samples were taken right after the dry summer season, however, it is expected that water volumes will be present again at winter season as it has happened before [41] and redilution of pollutants from the sediment sink is possible. Toxicity was generally low with sediment sample Number 1 exhibiting maximum values for all three organisms. This was accompanied by the highest conductivity (4.60 mS/cm) and TOC (28.66 mg/L) value. The rest of samples did not exceed 10% toxicity for all tested organisms. *Daphnia* was not negatively affected by the leachates, a fact corroborated by actual zooplankton structure findings in the lake in earlier years, where *Daphnia* species were predominant [41]. However, its contribution to zooplankton community was restricted to bigger individuals, with *Daphnia* neonates conspicuously missing. Three years earlier than the study of Michaloudi and Kostecka, toxicity to *Daphnia* in early-late summer was extremely high; especially, within the lake rather than in the receiving effluents, with 48 h toxicity reaching 50–90% of the samples. This toxicity was accompanied by high COD values (900–1,342 mg/L) and considerable alkalinity of the water (pH 10.3–10.6) [14]. In the present research, toxicity to all trophic chain representatives was low pointing to a low redilution potential from the sediment, at least under the conditions of the EN 12457-2 method. Nevertheless, the sediment basin of Koronia does act as a heavy metal (Zn, Cd, Cr, Pb, Mn, Cu, and Fe) sink as shown in relevant studies [42]. In any case, the ecological situation of Koronia is so dire that bioassays are of complementary value; the water balance is critical and the lake is disappearing fast. Furthermore, the food web of the lake is perturbed with complete lack of fish, presence of parasitic microscopic eukaryotes [39], and simplified invertebrate community with low biodiversity [41]. An effective and radical management scheme in the context of both water pollution elimination and water balance sustainability should be adopted, before the complete eradication of the lake.

Karla Lake exhibits a more typical situation of a Greek vulnerable ecosystem; it is a shallow lake ecosystem undergoing reconstruction phase [43]. Although Lake Karla is listed in the network of the Greek protected areas, as it is considered a vital aquatic ecosystem, in terms of biodiversity (it is a Natura site GR1420004 and a Special Protected Area site for birds) it is affected by both agricultural and industrial land uses in the surrounding area, acting as a sink of fertilizers, and surface run-off from the surrounding cultivated fields [44]. It is noted that micronutrients in the surface water caused a significant (>10%) induction of growth of *P. subcapitata*. This characteristic is indicative of high-organic burden of the lake, which in the long run may lead to eutrophic conditions. Nitrates were indeed elevated (20 mg/L) and conductivity was also considerable (3.85 mS/cm). The latter parameter renders the water of the lake unacceptable for drinking purposes [5]. Previous research in the last decade on the same lake concluded that the basin undergoes progressive eutrophication procedures which aid lengthy cyanobacterial blooms. Extensive blooms dominated by toxin producing species were recorded in the initial period of the lake's rehabilitation (2009–2011), which led to highly publicized fish kills [44]. Current research also shows that eutrophication is present with considerable amounts of microcystins [45]. The apparent eutrophication noted here and the aforementioned studies and the algal blooms encountered pose a significant risk on both public health and ecosystem biodiversity. The present situation is in marked contrast with previous results of the same area where *P. subcapitata* growth was minimal and it was only restored to control values with the addition of micronutrients [13]. Regarding bacteria and invertebrates, the toxic effects of the lake water were minimal in the present findings. Toxic effects in low trophic scale organisms were also minimal in previous research (1998) on Lake Karla where, out of 85 sampling points, maximum toxicity to *Daphnia* was only 30 with 78% of samples exhibiting toxicity lower than 10% [13]. The toxicity noted was attributed by the researchers mostly to the increased salinity of the lake and to possible presence of agrochemicals from the vicinity of agricultural plains. In summary, based on ecotoxicological assays, the quality of Karla Lake has worsened within a 15-year time; this deterioration is mostly due to eutrophication and periodical algal blooms while salinity- and pollution-related toxicity remains stable.

5. Conclusions

The results show the current situation of two vulnerable lake ecosystems under remediation by means of physicochemical measurements and ecotoxicity bioassays. Despite numerous efforts, the water quality of both ecosystems is deteriorating with Karla Lake exhibiting indications of increased eutrophication and Koronia Lake being plagued by culminating water deficit, increasing water pollution, and encumbered water quality. Results on treated wastewater samples in the same geographical region showed satisfactory performance, however, bioassays were capable of revealing different potential risks; in the case of Tirnavos, toxicity to certain trophic level representatives was still present whereas in the case of Lagadas,

nitrate burden may aid eutrophication of the receiving water in the long run. A battery of bioassays on organisms of different trophic levels is therefore indispensable, since it gives a rapid and comprehensive picture of the toxic potential of freshwater and wastewater samples.

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References

[1] G.A. Codd, Cyanobacterial toxins, the perception of water quality, and the prioritisation of eutrophication control, Ecol. Eng. 16 (2000) 51–60.
[2] World Water Assessment Programme. Available from: http://www.unesco.org (accessed July 2013).
[3] B.T. De Stasio Jr., D.K. Hill, J.M. Kleinhans, N.P. Nibbelink, J.J. Magnuson, Potential effects of global climate change on small north-temperate lakes: Physics, fish, and plankton, Limnol. Oceanogr. 41 (1996) 1136–1149.
[4] A. Ricciardi, R.J. Neves, J.B. Rasmussen, Impending extinctions of North American freshwater mussels (Unionoida) following the zebra mussel (*Dreissena polymorpha*) invasion, J. Anim. Ecol. 67 (1998) 613–619.
[5] Council Directive 98/83/EC of 3 November 1998 on the quality of water intended for human consumption.
[6] U.S. Environmental Protection Agency, EPA/625/R-04/108, Guidelines for Water Reuse, U.S. Environmental Protection Agency, Washington, DC, 2004.
[7] Environment Agency, Regulatory Whole Sample Ecotoxicological Testing: Comparison of Approaches, vol. 4, Environment Agency, Rotherham, 1995.
[8] D. Wei, A. Kisuno, T. Kameya, K. Urano, A new method for evaluating biological safety of environmental water with algae, *Daphnia* and fish toxicity ranks, Sci. Total Environ. 371 (2006) 383–390.
[9] H. Okamura, M. Piao, I. Aoyama, M. Sudo, T. Okubo, M. Nakamura, Algal growth inhibition by river water pollutants in the agricultural area around Lake Biwa, Japan, Environ. Pollut. 117 (2002) 411–419.
[10] J. Mankiewicz-Boczek, G. Nałęcz-Jawecki, A. Drobniewska, M. Kaza, B. Sumorok, K. Izydorczyk, M. Zalewski, J. Sawicki, Application of a microbiotests battery for complete toxicity assessment of rivers, Ecotoxicol. Environ. Saf. 71 (2008) 830–836.
[11] P. Palma, L. Ledo, S. Soares, I.R. Barbosa, P. Alvarenga, Integrated environmental assessment of freshwater sediments: A chemical and ecotoxicological approach at the Alqueva reservoir, Environ. Geochem. Health 36 (2013) 209–223.
[12] C.S. Laspidou, I. Kakoulidis, A. Loukas, Ecosystem simulation modeling of nitrogen dynamics in the restored lake Karla in Greece, Desalin. Water Treat. 33 (2011) 61–67.
[13] A. Angelaki, M. Sakellariou, D. Pateras, A. Kungolos, Assessing the quality of natural waters in Magnesia prefecture in Greece using Toxkits, in: G. Persoone, C. Janssen, W. De Coen (Eds.), New Microbiotests for Routine Toxicity and Biomonitoring, Kluwer Academic/Plenum Publishers, New York, NY, 2000, pp. 281–288.
[14] A. Kungolos, P. Samaras, V. Kimeroglou, X. Dabou, G.P. Sakellaropoulos, Water quality and toxicity assessment in Koronia Lake—Greece, Fresenius Environ. Bull. 7 (1998) 615–622.
[15] APHA-AWWA-WEF, Standard Methods for the Examination of Water and Wastewater, 20th ed., American Public Health Association, Washington, DC, 1999.
[16] CEN, Characterization of Waste—Leaching-compliance Test for Leaching of Granular Waste Material and Sludge—Part 2: One Stage Batch Test at a Liquid to Solid Ratio of 10 L/kg with Article Size below 4 mm (Without or with Size Reduction), EN12457-2´, European Committee for Standardization, Brussels, 2002.
[17] OECD Guideline 201 for Testing of Chemicals, Alga, Growth Inhibition Test, 1984
[18] OECD Guideline 202 for Testing of Chemicals, *Daphnia* sp., Acute Immobilisation Test and Reproduction Test, 1984.
[19] ISO 6341, Water quality—Determination of the inhibition of the mobility of *Daphnia magna* Straus (Cladocera, Crustacea)—Acute toxicity test, 1996.
[20] World Health Organization, WHO Health Guidelines for the Use of Wastewater in Agriculture and Aquaculture, Report of a WHO Scientific Group, World Health Organization, Geneva, 1989.
[21] Food and Agriculture Organization, Wastewater Treatment and Use in Agriculture, Irrigation and Drainage Paper 47, M.B. Pescod, Rome, 1992.
[22] S. Bakopoulou, C. Emmanouil, A. Kungolos, Assessment of wastewater effluent quality in Thessaly region, Greece, for determining its irrigation reuse potential, Ecotoxicol. Environ. Saf. 74 (2011) 188–194.
[23] Joint Ministerial Decree 145116/2011 regarding "the definition of measures, terms and processes regarding the reclamation of wastewater treatment", 2011 (in Greek).
[24] B. Ferrari, C.M. Radetski, A.M. Veber, J.F. Ferard, Ecotoxicological assessment of solid wastes: A combined liquid and solid phase testing approach using a battery of bioassays and biomarkers, Environ. Toxicol. Chem. 18 (1999) 1195–1202.
[25] V.K. Tyagi, A.K. Chopra, N.C. Durgpal, A. Kumar, Evaluation of *Daphnia magna* as an indicator of toxicity and treatment efficacy of municipal sewage treatment plant, J. Appl. Sci. Environ. Manage. 11 (2007) 61–67.
[26] R. Rojíčková, B. Maršálek, Selection and sensitivity comparisons of algal species for toxicity testing, Chemosphere 38 (1999) 3329–3338.
[27] V. Aruoja, Algae *Pseudokirchneriella subcapitata* in environmental hazard evaluation of chemicals and synthetic nanoparticles, PhD thesis, Estonian University of Life Sciences, Tartu, 2011.
[28] D. De Zwart, W. Slooff, The Microtox as an alternative assay in the acute toxicity assessment of water pollutants, Aquat. Toxicol. 4 (1983) 129–138.

[29] C.R. Arias-Barreiro, H. Nishizaki, K. Okubo, I. Aoyama, I.C. Mori, Ecotoxicological characterization of tannery wastewater in Dhaka, Bangladesh, Environ. Biol. 31 (2010) 471–475.
[30] H. Selçuk, G. Eremektar, S.J. Meriç, The effect of pre-ozone oxidation on acute toxicity and inert soluble COD fractions of a textile finishing industry wastewater, J. Hazard. Mater. 137 (2006) 254–260.
[31] J. Žaltauskaitė, R. Vaisiūnaitė, Evaluation of municipal effluent toxicity using higher plants and invertebrates, Environ. Res. Eng. Manage. 3 (2010) 17–23.
[32] E. Mendonça, A. Picado, S.M. Paixão, L. Silva, M. Barbosa, M.A. Cunha, Ecotoxicological evaluation of wastewater in a municipal WWTP in Lisbon area (Portugal), Desalin. Water Treat. 51 (2013) 4162–4170.
[33] L. Manusadžianas, L. Balkelyte, K. Sadauskas, I. Blinova, L. Põllumaa, A. Kahru, Ecotoxicological study of Lithuanian and Estonian wastewaters: Selection of the biotests, and correspondence between toxicity and chemical-based indices, Aquat. Toxicol. 63 (2003) 27–41.
[34] M.D. Hernando, A.R. Fernandezalba, R. Tauler, D. Barcelo, Toxicity assays applied to wastewater treatment, Talanta 65 (2005) 358–366.
[35] S.G. Schrank, H.J. José, R.F. Moreira, H.F. Schröder, Comparison of different advanced oxidation process to reduce toxicity and mineralisation of tannery wastewater, Water Sci. Technol. 50 (2004) 329–334.
[36] G.E. Marchant, Legal criteria and judicial precedents relevant to incorporation of hormesis into regulatory decision-making, Sci. Total Environ. 288 (2002) 141–153.
[37] L.N. Biggs, An investigation of hormesis using the Microtox assay, MSc thesis, University of Illinois, Urbana, 1994.
[38] V. Manakou, P. Tsiakis, A. Kungolos, A mathematical programming approach to restore the water balance of the hydrological basin of Lake Koronia, Desalin. Water Treat. 51 (2013) 2955–2976.
[39] V. Tsiridis, P. Samaras, A. Kungolos, G.P. Sakellaropoulos, Application of leaching tests for toxicity evaluation of coal fly ash, Environ. Toxicol. 21 (2006) 409–416.
[40] S. Genitsaris, K.Ar. Kormas, M. Moustaka-Gouni, Microscopic eukaryotes living in a dying lake (Lake Koronia, Greece), FEMS Microbiol. Ecol. 69 (2009) 75–83.
[41] A. Michaloudi, M. Kostecka, Zooplankton of Lake Koroneia (Macedonia, Greece), Biol. Brat. 59 (2004) 165–172.
[42] N. Gantidis, M. Pervolarakis, K. Fytianos, Assessment of the quality characteristics of two lakes (Koronia and Volvi) of N. Greece, Environ. Monit. Assess. 125 (2007) 175–181.
[43] C.S. Laspidou, V. Vaina, Ecosystem modeling of sediment dynamics in the constructed wetland Carla in Central Greece, Int. J. Des. Nat. Ecodyn. 3 (2009) 273–280.
[44] A. Oikonomou, M. Katsiapi, H. Karayanni, M. Moustaka-Gouni, K.A. Kormas, Plankton microorganisms coinciding with two consecutive mass fish kills in a newly reconstructed lake, Sci. World J. 2012 (2012) 504135, doi: 10.1100/2012/504135.
[45] Th. Papadimitriou, M. Katsiapi, K.Ar. Kormas, M. Moustaka-Gouni, I. Kagalou, Artificially-born "killer" lake: Phytoplankton based water quality and microcystin affected fish in a reconstructed lake, Sci. Total Environ. 452–453 (2013) 116–124.