Evaluation of the Distribution of Heavy Metals and Arsenic in Inland Wetlands (Peru) Using Multivariate Statistical Methods

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ABSTRACT
Heavy metals in the environment circulate among the different compartments and can accumulate, convert into organic complexes and biomagnify in the food chain. The objective of this study was to evaluate the distribution of heavy metals and arsenic in inland wetlands using multivariate statistical methods. Samples of water, sediment and aquatic vegetation were collected at 48 sampling sites established in the Paca and Tragadero lagoon wetlands. The determination of heavy metals and arsenic was carried out using the flame atomic absorption spectrophotometry method. The decreasing order of heavy metal and arsenic concentration in sediment was Fe>Zn>Pb>As, in water it was Zn>Fe>Pb>As and in S. californicus it was Zn>Fe>Pb>As. Redundancy analysis (RDA) indicated that there are significant differences in heavy metal and arsenic concentrations in sediment between ponds and between sampling sites. Generalized linear model (GLM) analysis on vegetables indicated that the concentrations of heavy metals and arsenic in the vegetable increase as a function of their concentration in sediment.

Keywords: heavy metals, arsenic, indicators, wetlands, freshwater, sediment, sediment, Schoenoplectus californicus.

INTRODUCTION
The water quality of inland aquatic systems has been a major concern in recent decades, due to the increasing deterioration they are experiencing. Contamination of the aquatic environment by heavy metals is one of the main disturbances, as it not only affects the physical environment but also the functioning of ecosystems (Rizo-Patrón et al., 2013). Heavy metals in the environment circulate between different compartments and enter the food chain and can reach toxic concentrations (Duinker et al., 2013). The presence of heavy metals in the environment is due to multiple factors such as erosion, atmospheric deposition, geological weathering, industrial processes, domestic waste, agricultural activities, and vehicle emissions.

Currently, heavy metals are of great significance as indicators of the ecological quality of any aquatic ecosystem due to their toxicity and bioaccumulative behavior. Heavy metals in the aquatic environment are trapped in sediments and constitute the secondary source of contamination of the water column (Custodio et al., 2019). They can accumulate in aquatic biota, convert to organic complexes and biomagnify in the food chain. Water quality degradation is a limiting factor in the availability of water for its various uses worldwide (Engin et al., 2017). Projections of future water scarcity in the tropical Andes of the world are increasingly alarming. In some Peruvian watersheds, conflicts have already arisen not only because of the problem of water availability, as is the case of the declining flow of the Santa River and the shrinking of alpine-type wetlands (Bury et al., 2013) but also because of the major alterations that anthropogenic pollutants have been causing in water quality (Ramani et al., 2014; Choudri and Baawain, 2014).

Peru is a country with great water potential, but like many other countries, it faces water availability problems due to the growth of population densification and pollution problems. Changes in aquatic systems and anthropogenic sources of pollution is multivariate and complex. Therefore,
the assessment of water quality requires a comprehensive understanding of the temporal and spatial behavior of water characteristics. It is necessary and important to have reliable information on the pressures that these systems are experiencing in order to direct management and pollution control strategies. Many studies report that multivariate statistical methods are effective for characterizing and evaluating water quality and interpreting spatio-temporal variations of water quality indicators. In this sense, the present study aimed to evaluate the distribution of heavy metals and arsenic in inland wetlands using multivariate statistical methods.

**MATERIALS AND METHODS**

**Study area**

The Paca (320 ha) and Tragadero (174 ha) lagoon wetlands are located in the Junin region, in the Central Andes of Peru, in the northeastern Mantaro Valley at 11º 46' 48" S and 75º 30' 13" N, at altitudes ranging from 3390 to 3460 m.a.s.l., respectively (Figure 1). Both lagoon systems have an area of submerged and emergent macrophyte cover dominated by cattails. The climate of the region is cold, with an average annual temperature of 11.4 °C and an annual precipitation of 649 mm, and the summer (January to March) is the wet season.

**Collection of water, sediment and aquatic vegetation samples**

Water samples were collected at 48 sampling sites on the shore and in the middle part of each lagoon, during the rainy season. The water samples were pre-treated with a 10% nitric acid solution for 24 hours and rinsed with double distilled water. Then, 1.5 ml of concentrated nitric acid was added to one liter of water from each sample for preservation (APHA/AWWA/WEF, 2012). Sediment samples were collected at the same collection sites as the water samples. Sediment sample collection was conducted using an Ekman-type dredge. Aquatic vegetation, represented by Schoenoplectus californicus, was collected using gardening tools. The samples as a whole (water, sediment and aquatic vegetation), under refrigerated conditions, were sent to the Water Research Laboratory of the National University of Central Peru for analytical determination.

**Determination of heavy metals and arsenic**

The sample was prepared by adding 250 ml of water in a beaker, which was brought to boiling point until 100 ml were obtained. Immediately, the destruction of the organic matter was carried out and again it was taken to boiling. It was allowed to cool and then 10 ml of distilled water was added, filtered, and leveled in a 100 ml flask with 1% nitric acid (APHA/AWWA/
The quantitative determination of heavy metals and arsenic was performed through the flame atomic absorption spectrophotometry method (Abril Díaz et al., 2010). Sediment samples were dried in a 105 °C oven. They were then subjected to acid digestion with nitric acid, followed by oxidation with hydrogen peroxide and finally with hydrochloric acid (Harwood & Stroud, 2012). The concentration of heavy metals was determined using a flame atomic absorption spectrometer. Working solutions were prepared from successive dilutions of the stock solutions with 1% (v/v) nitric acid. *Schoenoplectus californicus* samples were dried under shade and then pulverized in a stainless steel mill. The determination of heavy metals was performed from 0.5 g of aquatic vegetation in a 100 mL beaker. Then, 10 mL of nitric acid was added and allowed to stand for half an hour for the disintegration of the organic matter, then boiled for five minutes until a pasty consistency was obtained. It was gauged with distilled water and 10 ml of hydrochloric acid was added and it was filtered.

### Data analysis

To test the relationship between element concentrations in water and sediment with element concentrations in plant tissues, a redundancy analysis (RDA) was performed by means in Canoco V5 software, RDA is used in ecology for ordination by direct gradient analysis, where a matrix of response variables, Y, is analyzed with respect to a corresponding matrix of environmental variables, X (Gan et al., 2017). RDA can be described as a series of multiple regressions followed by principal component analysis. Here, instead of a vector for a Y variable, a data matrix with many Y variables is analyzed. Each vector variable, Y, is regressed on the matrix of environmental variables corresponding to the dependent variables (Legendre & Andersson, 1999). To quantify the different absorption capacity of *Schoenoplectus californicus* leaves in relation to the sediment, transfer factors resulting from the quotient of the means of the element concentrations in the leaf and sediment were calculated (Peris et al., 2007).

The generalized linear model (GLM) was used as a flexible generalization of ordinary linear regression that allows for response variables that have error distribution patterns other than a normal distribution. The GLM generalizes linear regression by allowing the linear model to be related to the response variable (plant tissue metal concentration) through an appropriate link function and allows the magnitude of the variance of each measurement to be a function of its predicted value (McCulloch and Neuhaus, 2014).

### RESULTS AND DISCUSSION

The statistics of heavy metal and arsenic concentrations in sediment, water and aquatic vegetation are shown in Table 1. In sediment, the decreasing order of concentration was Fe>Zn>Pb>As. The lowest mean concentrations of Pb (45.75 mg/kg), Fe (9531 mg/kg) and As (13.66 mg/kg) were recorded in the Paca lagoon; while those of Zn in the Tragadero lagoon (71.59 mg/kg). Sediment Pb and As concentrations in both aquatic environments exceeded the provisional sediment quality guidelines (30.20 mg/Kg and 7.24 mg/Kg, respectively) stipulated by the Canadian Council of Ministers of the Environment (CCME, 2007). However, the concentrations of the four toxic metals evaluated did not exceed the probable effect concentration (PEC) threshold values reported by MacDonald et al. (2000). In water, the decreasing order of concentration was Zn>Fe>Pb>As. The lowest mean concentrations of Pb (0.012 mg/L), Fe (0.022 mg/L) and As (0.004 mg/L) were recorded in the Paca lagoon; while those of Zn in the Tragadero lagoon (0.079 mg/L). Pb concentrations recorded in water from both lagoons exceeded the environmental quality standards for water (EQSw) (0.0025 mg/L) of the Peruvian standard (Ministry of the Environment, 2017) and WHO (0.01 mg/L) (WHO, 2011). Meanwhile, the concentrations of Zn, Fe and As were lower than the national and international regulatory limits. In *S. californicus*, the decreasing order of concentration was Zn>Fe>Pb>As. The lowest mean concentrations of Pb (0.012 mg/L), Fe (0.022 mg/L) and As (0.004 mg/L) were recorded in the Paca lagoon; while those of Zn in the Tragadero lagoon (0.079 mg/L). Pb concentrations recorded in water from both lagoons exceeded the environmental quality standards for water (EQSw) (0.0025 mg/L) of the Peruvian standard (Ministry of the Environment, 2017) and WHO (0.01 mg/L) (WHO, 2011). Meanwhile, the concentrations of Zn, Fe and As were lower than the national and international regulatory limits. In *S. californicus*, the decreasing order of concentration was Zn>Fe>Pb>As. The lowest mean concentrations of Pb (0.012 mg/L), Fe (0.022 mg/L) and As (0.004 mg/L) were recorded in the Paca lagoon; while those of Zn in the Tragadero lagoon (0.079 mg/L). Pb concentrations recorded in water from both lagoons exceeded the environmental quality standards for water (EQSw) (0.0025 mg/L) of the Peruvian standard (Ministry of the Environment, 2017) and WHO (0.01 mg/L) (WHO, 2011). Meanwhile, the concentrations of Zn, Fe and As were lower than the national and international regulatory limits. In *S. californicus*, the decreasing order of concentration was Zn>Fe>Pb>As.

Redundancy analysis of correlations between metal concentrations in *S. californicus* and environmental factors are presented in the Biplot (Figure 2). Figure 2A shows correlation between the distribution matrices of metals found in the sediment and the concentration matrix of metals found in the leaf. The eigenvalues for the first and second coordinates were 0.58 and 0.0874 (Table 2), revealing that the explained variation can be
interpreted as a function of the first common axis with 58.45% of explained variation. The second axis presented a low level of explained variation of 8.74%, a minimum value, but not unimportant, since it explains the internal distribution in lentic environments affected by different anthropogenic activities. The RDA analysis indicated that there are significant differences in the concentrations of toxic metals in sediment between ponds and between sampling sites. The analysis shows that the heavy metals and arsenic in sediment that significantly differentiate Paca and Tragadero lagoons are Fe and As. While, the metal in S. californicus that significantly differentiates it is Pb.

Zn concentrations in both ponds in both sediment and leaf denote variation generated by anthropogenic activities around each pond. The direct selection redundancy analysis in Table 2 indicates that As concentrations present in the sediment would correlate significantly and positively with As and Pb concentrations in the plant (explained % similarity in matrix distribution of 41.7%). The correspondence of Pb with As in the leaf tends to be low, indicating that As in sediment functions as a synergistic element of Pb concentrations in the leaf. However, leaf As concentrations are not affected by sediment As concentrations, as this element tends to accumulate in roots. In addition, Zn and Pb concentrations in sediment would correlate to a lesser degree with Zn concentrations in S. californicus (explained % matrix similarity of 16.5 and 8.9% for Zn and Pb, respectively), since as indicated in Figure 2A, these values would tend to correlate with the second axis.

Table 1. Concentrations of heavy metals and arsenic in sediment, water and Schoenoplectus californicus in inland wetlands

| Element | Lagoon   | Sediment (mg/Kg) n = 48 | Water (mg/L) n = 48 | S. californicus (mg/Kg) n = 48 |
|---------|----------|-------------------------|---------------------|--------------------------------|
|         |          | Mean ± SD | Min - Max | Mean ± SD | Min - Max | Mean ± SD | Min - Max |
| Pb      | Paca     | 45.795 ± 4.106 (35.55 - 53.96) | 0.012 ± 0.002 (0.007 - 0.016) | 0.01 ± 0.005 (0.004 - 0.02) |
|         | Tragadero| 49.71 ± 5.53 (41.52 - 59.52)  | 0.019 ± 0.005 (0.009 - 0.026) | 0.034 ± 0.006 (0.026 - 0.047) |
| Zn      | Paca     | 85.16 ± 11.95 (67.62 - 105.52) | 0.08 ± 0.004 (0.074 - 0.086) | 0.13 ± 0.032 (0.083 - 0.177) |
|         | Tragadero| 71.59 ± 6.71 (62.62 - 84.64)  | 0.079 ± 0.008 (0.064 - 0.092) | 0.19 ± 0.068 (0.121 - 0.356) |
| Fe      | Paca     | 9531 ± 764 (7784 - 10875)  | 0.022 ± 0.002 (0.018 - 0.024) | 0.016 ± 0.004 (0.01 - 0.026) |
|         | Tragadero| 17170 ± 4340 (10659 - 21649) | 0.039 ± 0.005 (0.028 - 0.046) | 0.027 ± 0.011 (0.015 - 0.049) |
| As      | Paca     | 13.669 ± 1.982 (10.35 - 17.534) | 0.004 ± 0.001 (0.002 - 0.006) | 0.013 ± 0.003 (0.008 - 0.018) |
|         | Tragadero| 22.988 ± 3.64 (16.21 - 28.828) | 0.022 ± 0.002 (0.018 - 0.025) | 0.015 ± 0.003 (0.011 - 0.02) |

Figure 2. Redundancy analyses of the correlations between metal contents in vegetables (green lines) and sediment properties variables (A, red dash lines) and waters (B, blue dash lines)
Figure 2B shows the correlation between the matrices of the distribution of metals found in the water and the matrix of the concentration of metals found in the leaf. The eigenvalues for the first and second coordinates were 0.7416 and 0.026 (Table 4), indicating that the explained variation can be interpreted as a function of the first common axis with 74.9% of variation resulting in a high range of similarity. The second axis presented a low level of explained variation of 2.6%. The RDA analysis indicates that there are significant differences in the concentrations of toxic metals in water between ponds and sampling sites. The analysis shows that the metals in water that significantly differentiate the Paca and Tragadero ponds are Pb and Zn, while the metal in the plant that significantly differentiates it is Zn.

Zn concentrations in both water and leaf of *S. californicus* determine variation between sampling sectors. The direct selection redundancy analysis in Table 3 indicates that the Zn concentration present in water would not correlate significantly and positively with Zn concentrations in the plant (explained % similarity in matrix distribution of 40.2%), indicating that the Zn concentrations found in the leaf are due to the effect of the concentrations in the sediment and not the water as shown by the significant correspondence of Zn contribution for the second axis with 23.9% in Table 2. Whereas, the Pb concentrations in the leaf are given by the Pb concentrations in the water with a significant contribution of 49.6% for the first axis.

Generalized linear model (GLM) analyses were performed for the studied toxic metals in plants based on the concentration of the metal in sediment. The results of the analyses indicated the impact of Pb in sediment on Pb content in *S. californicus* leaves (Figure 3A). The adjusted explained variance was low (12.7%), denoting that the similarity in the articulation of the distribution matrices of the observations for Pb was low, understood as a functional model of medium quality. The p-value (prob > F) was less than 0.05, indicating that there is significant linear growth, but with a medium quality model. The analysis indicated that the concentrations in the plant increase as a function of its concentration in the sediment,

### Table 2. Distance-based redundancy analysis (RDA) between sediment element concentration matrices and *Schoenoplectus californicus*. Total variation is 38.52, explanatory variables account for 68.9% (adjusted explained variation is 66.1%)

| Statistics       | Axis 1    | Axis 2     | Axis 3 | Axis 4 |
|------------------|-----------|------------|--------|--------|
| Eigenvalues      | 0.5845    | 0.0874     | 0.0153 | 0.0023 |
| Explained variation (cumulative) | 58.45     | 67.19      | 68.71  | 68.94  |

| RDA Forward selection | Explains % | Contribution % | pseudo-F | P    |
|-----------------------|------------|----------------|----------|------|
| As                    | 41.7       | 60.5           | 32.9     | 0.002|
| Zn                    | 16.5       | 23.9           | 17.8     | 0.002|
| Pb                    | 8.9        | 13             | 12       | 0.002|
| Fe                    | 1.8        | 2.6            | 2.4      | 0.082|

### Table 3. Distance-based redundancy analysis (RDA) between toxic metal concentration matrices in water and *Schoenoplectus californicus*, Total variance is 0.19, explanatory variables account for 77.1% (adjusted explained variance is 74.9%)

| Statistics       | Axis 1    | Axis 2    | Axis 3 |
|------------------|-----------|-----------|--------|
| Eigenvalues      | 0.7416    | 0.0269    | 0.0022 |
| Explained variation (cumulative) | 74.16     | 76.86     | 77.08  |

| RDA Forward selection | Explains % | Contribution % | pseudo-F | P    |
|-----------------------|------------|----------------|----------|------|
| Pb                    | 38.2       | 49.6           | 28.4     | 0.002|
| Zn                    | 31         | 40.2           | 45.2     | 0.002|
| Fe                    | 5.3        | 6.9            | 9.2      | 0.002|
| As                    | 2.6        | 3.3            | 4.8      | 0.032|
corroborating the trend of linear increase of the GLM analysis. The concentrations of Pb in the leaf of the plant increase as the concentration in the sediment increases, understanding that S. californicus is a good bioaccumulator of Pb, not having a saturation in relation to the concentration of what is found in the sediment, even exceeding values of 0.04 mg/kg in leaf for a concentration higher than 58 mg/kg in sediment.

The results of the analyses for Zn in Figure 3B explain the impacts of sediment Zn on the Zn content of S. californicus leaves. The adjusted explained variance is very low, with a value of 0.4%, denoting that the similarity in the articulation of the distribution matrices of the observations for Zn is null (poor quality functional model). The p-value (prob > F) was significantly higher than 0.05, expressing that there is no linear growth, since an absorption ceiling was reached in the leaf (between 0.1 and 0.2 mg/kg) of S. californicus. For Fe whose distribution is shown in Figure 3C, the adjusted explained variation was found to be very low, with a value of 6.1%, denoting that the similarity in the articulation of the distribution matrices of the observations for Fe is low. The p-value (prob > F) was significantly higher than 0.05, indicating a cap on uptake by the leaf, regardless of the concentration in the sediment, being between 0.01 and 0.02 mg/kg.

CONCLUSIONS

The concentrations of heavy metals and arsenic in sediment, water and S. californicus from

![Figure 3](image-url). Generalized linear model (GLM) equation models of Pb (A), Zn (B), Fe (C) and As (D) contents in Schoenoplectus californicus leaves and sediment.
in the central Andes of Peru were determined. In sediment, the decreasing order of concentration was Fe>Zn>Pb>As. The lowest mean concentrations of Pb (45.75 mg/kg), Fe (9531 mg/kg) and As (13.66 mg/kg) were recorded in the Paca lagoon; while those of Zn in the Tragadero lagoon (71.59 mg/kg). In water, the decreasing order of concentration was Zn>Fe>Pb>As. The lowest mean concentrations of Pb (0.012 mg/L), Fe (0.022 mg/L) and As (0.004 mg/L) were recorded in the Paca lagoon; while those of Zn in the Tragadero lagoon (0.079 mg/L). In S. californicus, the decreasing order of concentration was Zn>Fe>Pb>As. The lowest mean concentrations of Pb (0.010 mg/kg), Zn (0.125 mg/kg), Fe (0.016 mg/kg) and As (13.66 mg/kg) and Zn (0.013 mg/kg) were recorded in the Paca lagoon. RDA analysis revealed that Fe and As in sediment and Pb in S. californicus significantly differentiate Paca and Tragadero lagoons. Analysis of the behavior of heavy metals and arsenic in the Paca and Tragadero wetlands with the generalized linear model (GLM) indicated the impact of Pb in sediment on the Pb content in the leaves of Schoenoplectus californicus. Leaf Pb concentrations of S. californicus increase with increasing sediment concentration, revealing that S. californicus is a good Pb bioaccumulator.

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REFERENCES

1. Diaz, A.N., Ruiz, B.J.A., Reyes, F.E., Cejudo, G.A., Novo, J.J., Peinado, P.J., Meléndez-Valdés, F., & Túnez Fiñana, I. 2010. Espectrofotometria: Espectros de absorción y cuantificación colorimétrica de biomoléculas. Campus Universitario de Rabanales. Departamento de Bioquímica y Biología Molecular, 1, 1–8. Retrieved from http://www.ucu.es/dptos/bioquimica-biol-mol/pdfs/08_espectrofotometria.pdf
2. APHA/AWWA/WEF, 2012. Standard Methods for the Examination of Water and Wastewater. Standard Methods. https://doi.org/ISBN 9780875532356
3. Bury, J., Mark, B.G., Carey, M., Young, K.R., McKenize, J.M., Baraer, M., Francés A., Molly, H., & Polk, M.H. 2013. New geographies of water and climate change in Peru: Coupled natural and social transformations in the Santa River watershed. Annals of the Association of American Geographers, 103(2), 363–374. https://doi.org/10.1080/00045608.2013.754665
4. CCME, 2007. Canadian water quality guidelines for the protection of aquatic life. Canadian water quality guidelines.
5. Choudri, B.S., & Baawain, M. 2014. Effects of Pollution on Freshwater Organisms. Water Environmental Research, 86(10), 1832–1868. https://doi.org/10.2175/106143014X14031280668452
6. Custodio, M., Miranda, G., Peñaloza, R., De la Cruz, H., & Channé, F. 2019. Variability of the water quality characterizing high andean lagoons for tourist use evaluated through multivariate statistical methods, Junin, Peru. Journal of Ecological Engineering, 20(8), 1–11. https://doi.org/10.12911/22998993/109800
7. Duinker, P.N., Burbidge, E.L., Boardley, S.R., & Greig, L.A. 2013. Scientific dimensions of cumulative effects assessment: toward improvements in guidance for practice. Environmental Reviews, 21(1), 40–52. https://doi.org/10.1139/er-2012-0035
8. Engin, M.S., Uyanik, A., & Cay, S. 2017. Investigation of trace metals distribution in water, sediments and wetland plants of Kızılırmak Delta, Turkey. International Journal of Sediment Research, 32(1), 90–97. https://doi.org/10.1016/j.jirs.2016.03.004
9. Ferreira, J.G., Andersen, J.H., Borja, A., Bricker, S. B., Camp, J., Cardoso, M., … Claussens, U. 2011. Estuarine, Coastal and Shelf Science Overview of eutrophication indicators to assess environmental status within the European Marine Strategy Framework Directive. Estuarine, Coastal and Shelf Science, 93(2), 117–131. https://doi.org/10.1016/j.ecss.2011.03.014
10. Gan, Y., Wang, L., Yang, G., Dai, J., Wang, R., & Wang, W. 2017. Multiple factors impact the contents of heavy metals in vegetables in high natural background area of China. Chemosphere, 184(27), 1388–1395. https://doi.org/10.1016/j.chemosphere.2017.06.072
11. Harwood, J.J., & Stroud, R.A. 2012. A survey on the utility of the USEPA CADDIS stressor identification procedure. Environmental Monitoring and Assessment, 184(6), 3805–3812. https://doi.org/10.1007/s10614-011-2225-1
12. Legendre, P., & Andersson, M. 1999. Distance-based redundancy analysis: Testing multispecies responses in multifactorial ecological experiments. Ecological Monographs, 69(1), 1–24.
13. MacDonald, D.D., Ingersoll, C.G., & Berg, T.A. 2000. Development and evaluation of
consensus-based sediment quality guidelines for freshwater ecosystems. Archives of Environmental Contamination and Toxicology, 39(1), 20–31. https://doi.org/10.1007/s002440010075
14. McCulloch, C., & Neuhaus, J. 2014. Generalized Linear Mixed Models. Wiley Stats Ref, 1–8. https://doi.org/10.4135/9781506326139.n286
15. Ministry of the Environment, 2017. DS No 004-2017-MINEN, Environmental Quality Standards for Water (EQS-Water). El Peruano. Lima, Peru.
16. Peris, M., Micó, C., Recatalá, L., Sánchez, R., & Sánchez, J. 2007. Heavy metal contents in horticultural crops of a representative area of the European Mediterranean region. Science of the Total Environment, 378(1–2), 42–48. https://doi.org/10.1016/j.scitotenv.2007.01.030
17. Ramani, S., Dragun, Z., Kapetanović, D., Kostov, V., Jordanova, M., Erk, M., & Hajrulai-Musliu, Z. 2014. Surface water characterization of three rivers in the lead/zinc mining region of northeastern Macedonia. Archives of Environmental Contamination and Toxicology, 66(4), 514–528. https://doi.org/10.1007/s00244-014-0012-z
18. Rizo-Patrón V.F., Kumar, A., McCoy Colton, M.B., Springer, M., & Trama, F.A. 2013. Macroinvertebrate communities as bioindicators of water quality in conventional and organic irrigated rice fields in Guanacaste, Costa Rica. Ecological Indicators, 29, 68–78. https://doi.org/10.1016/j.ecolind.2012.12.013
19. Wenn, C.L. 2008. Do freshwater macroinvertebrates reflect water quality improvements following the removal of point source pollution from Spen Beck, West Yorkshire. Earth & Environment, 3, 369–406. Retrieved from http://homepages.see.leeds.ac.uk/~lecac/ejournal/3,369-406.pdf
20. WHO, 2011. Water quality for drinking: WHO guidelines. (World Health Organization, Ed.), WHO (4th ed.). https://doi.org/10.1007/978-1-4020-4410-6_184