Chilean J. Agric. Anim. Sci., ex Agro-Ciencia (2023) 39(2):210-216.
https://doi.org/10.29393/CHJAA39-18ONJD30018

ISSN 0719-3890 online

OPTIMIZATION OF NEUTRALIZING POWER OF *Mytilus chilensis* SEASHELLS IN ACID ALLUVIAL SOIL OF ÑUBLE COAST

Javier Leiva-Vega[1]*, Carolina Shene[2], and Danela Silva-Ferrer[3]

[1] Centre for Biotechnology and Bioengineering (CeBIB), Universidad de la Frontera, Avenida Francisco Salazar 1141, Temuco, Chile
https://orcid.org/0000-0001-7792-0065
[2] Chemical Engineering Department, Universidad de La Frontera, Avenida Francisco Salazar 1141, Temuco, Chile
https://orcid.org/0000-0003-4646-038X
[3] Food Engineering Department, Universidad del Bío-Bío, Avenida Andrés Bello 720, Chillán, Chile
https://orcid.org/0009-0003-5793-5624
* Corresponding author: javier.leiva.vega@gmail.com

ABSTRACT

Seashells, residues from mussel (*Mytilus chilensis*), Hupé 1854 processing, are an environmental load on the coasts of southern Chile. The main component of this waste is calcium carbonate, which is commonly used to neutralize soil acidity. Its application in meadows is limited due to the large quantities (tons) required. The objectives of this work were to determine the most appropriate type of mussel seashells (*M. chilensis*) as a source of calcium carbonate ($CaCO_3$) for pH neutralization of an alluvial acidic soil collected from Ñuble Coast, and to identify its optimal dose. The seashell doses were optimized using response surface methodology (RSM) to achieve a soil pH close to neutral and a relatively high neutralizing power, which was defined in comparison to the pH increase achieved with commercial $CaCO_3$. Seashells with organic matter was the most suitable formulation for increasing soil pH with a high relative neutralizing power (~ 94%), requiring a dose of 23% (w/w) to achieve soil pH values close to neutral. The fitted quadratic model explains 98.9% of variation in experimental data. This work lays the foundations for future studies aimed to find new agricultural and/or livestock applications of wastes from the shellfish industry.

Keywords: Calcium carbonate, seashells, relative neutralizing power, response surface methodology.

INTRODUCTION

The growing demand for marine products has led to the increasing development of the shellfish industry. Mollusk aquaculture accounts for 42.6% of the world's aquatic production (in million tons). Chile is the world's fourth largest producer, with approximately 12.5% of the global aquatic production (Zhan et al., 2022). In mollusks, however, inedible calcareous seashells represent 70% of the product, and constitute a waste source that pollutes the marine coasts, affecting coastal ecosystems (Zhan et al., 2022). Therefore, seashell waste should be efficiently reused to promote sustainable development and the circular economy in agriculture and the shellfish industry. It is well known that the main component of seashells is $CaCO_3$, which can be a matter of interest in agriculture due to its low cost and availability (Egerić et al., 2019). However, as seashells remain in an ecosystem for a long time and have a low ability to metabolize pollutants

Received: February 23, 2023 Accepted: July 18, 2023

in water, they can be a potential source of heavy metals (Saleh et al., 2021).

Organic matter content and pH are some of the leading indicators of soil quality, which are related to biochemical processes associated with soil fertility (Bünemann et al., 2018). Soil pH affects the absorption of nutrients by the plant, where the established pH ranges are: 6.5 to 7.5 for phosphorus (P); 6 to 8 for nitrogen (N); > 6 for potassium (K) and sulfur (S); 7 to 8.5 for calcium (Ca) and magnesium (Mg); < 6 for iron (Fe); and 5 to 7 for copper (Cu) and zinc (Zn) (Goulding, 2016). Thus, acid soils limit the availability of P, Ca, and Mg (Egerić et al., 2019). Specifically, the Ñuble Coast alluvial soils are located next to the rivers that cross the Ñuble Region from the mountains to the sea; the soils are poor in organic matter, present moderate biological activity, and are slightly acidic (pH from 5.6 to 6.5). They are regularly poor in nitrogen, phosphorus, and potassium (Hirzel, 2020).

Using $CaCO_3$ from seashells in acid soils increases Ca content in the soil, and in turn increases the pH, achieving values close to neutral (Summa et al., 2022; Fernández-Calviño et al., 2017). However, if plants have excess calcium, nutrients will not be available and growth will cease or become slower (Ab Manan et al., 2018). In addition, most alkaline amendments can immobilize heavy metal contaminant particles and reduce their bioavailability (Lu et al., 2020).

Therefore, the objectives of this work were to determine the most appropriate type of mussel seashells, with or without organic residues, as source of $CaCO_3$ for the neutralization of the pH of an alluvial acidic soil collected from Ñuble Coast, and to identify its optimal dose.

MATERIALS AND METHODS

Collection and preparation of mussel seashells

Mussel seashells (*Mytilus chilensis),* Hupé 1854 as waste of the shellfish industry located in Dalcahue (Chiloé Island, Chile) were classified as seashells with organic matter (*W-OM*) and without organic matter (*Wo-OM*) residues after the removal of residual meat. First, the seashells were washed with tap water and dried in a tray dryer (Model 30-1060, Memmert GmbH + Co. KG, Schwabach, Germany) at 50 °C for 12 to 20 h until moisture content reached 1 - 20 % w/w. The seashells were ground using a hammer mill (Model M0LTN0H11, Plaspak, Santiago, Chile) with a fine sieve (1.7 mm opening). Heavy metal concentrations in seashells, including arsenic (As), cadmium (Cd), mercury (Hg) and lead (Pb), were analyzed by flame atomic absorption spectrometry (Caramatín-Soriano et al., 2021).

Obtaining and preparation of soil

The alluvial soil samples were collected in Buchupureo, Cobquecura, Coast of Ñuble Region (Chile), to a depth of approximately 20 cm and transported to the laboratory in plastic containers (Muñoz-Sepúlveda, 2020). Plant matter and stones were removed, and the clean soil was sieved using a 2 mm aperture sieve.

Moisture and pH measurements

Moisture and soil pH were measured with a digital soil meter (PH328, SMART SENSOR, Fujian, CHN). A digital meter is suitable for measuring moisture in the range of 1 to 84% (accuracy ± 4% moisture) and pH between 2.0 and 8.5 (accuracy ± 0.2 pH). The alluvial soil exhibited a moisture content of 23.42 ± 1.26% (wet basis), and an initial pH value of 5.76 ± 0.21 (considered approximately as pH 5.8).

Preparation of soil and mussel seashells mixture

Three formulations of mussel seashells were evaluated: (i) *W-OM*, (ii) *Wo-OM*, and (iii) a mixture of equal mass of *W-OM* and *Wo-OM* mussel seashells (*50*:*50*). The mussel seashells (from 1 to 25% w/w) and soil (from 75 to 99% w/w) mass were mixed until the samples were homogenized. In the pots, the moisture was adjusted to 28 - 32% (wet basis), which is recommended for this soil type (Ulloa-Jara, 2022), and then soil pH was measured. The control consisted of soil with the addition of high-purity calcium carbonate, 99.999% (Merck, Darmstadt, GE), applied at the same concentrations used for seashells.

Relative neutralizing power

The relative neutralizing power (RNP) of the mussel seashell formulations on the alluvial soil, was defined as the pH increase achieved with each formulation (pH formulation) with respect to the control (soil without any formulation; pH value ~ 5.8), divided by the increase achieved with commercial $CaCO_3$, used as reference (Hirzel et al., 2017). The $CaCO_3$ content in mussel seashells was determined using the acid-base titration method (Fu et al., 2020). The estimation was performed according to the following relationship:

$$\text{RNP (\%)} = \frac{\text{pH formulation} - \text{pH control}}{\text{pH } CaCO_3 - \text{pH control}} * 100 \qquad (1)$$

Modeling and optimization using RSM

The influence of mussel seashell formulation type and its dose on their RNP of the acid alluvial soil was studied by response surface methodology. The control variables were the formulation type (three levels: *W-OM*, *Wo-*

OM, *50:50*) and its dose in grams (thirteen levels: 1, 3, 5, 7, 9, 11, 13, 15, 17, 19, 21, 23 and 25 g mussel seashell per 100 g of mixture "seashell + soil"), together with soil pH resulting from the mixture "mussel seashell formulation + soil" (pH from 5.8 to 7.2). The response variable (Y) was RNP of mussel seashell formulations on an acidic alluvial soil. Experimental data was fitted to a second-order polynomial. The equation was expressed in terms of coded variables (X_1, mussel seashells doses; X_2, pH resulting from the mixture "seashell + soil") using the following relationship:

$$Y= \beta_0+ \beta_1X_1 + \beta_2X_2 + \beta_{12}X_1X_2 + \beta_{11}X^2_1 + \beta_{22}X_2^2 \qquad (2)$$

where: β_0, is constant; and β_1, β_2, β_{12}, β_{11}, β_{22}, are regression coefficients.

Statistical analysis

The results were analyzed with Statgraphics Centurion XVI statistical software (Statistical Graphics Corp., Herdon, VA, USA).

RESULTS AND DISCUSSION

Optimization of the relative neutralizing power

The effectiveness of mussel seashell formulations for neutralizing the acidity of the alluvial soil is presented in Fig. 1. The formulations considered the *W-OM* and their removal (*Wo-OM*) after eliminating the residual mussel meat, and included a mixture of equal mass of *W-OM* and *Wo-OM*, denominated as *50:50* formulation.

The mussel seashells exhibited heavy metal contents (< 0.05 mg kg^{-1} As, 0.27 mg kg^{-1} Cd, < 0.05 mg kg^{-1}, 1.59 mg kg^{-1} Pb) lower than 5 mg kg^{-1}, which is the limit in fertilizers according to the Agricultural and Livestock (SAG) of Chile. Heavy metals exert toxic effects on soil biota, affecting key microbial processes and decreasing population size and activity of soil microorganisms (Singh and Kalamdha, 2011). Therefore, the low metal content observed in mussel seashells poses no threat to soil health and could allow free availability of nutrients to the crops.

The results showed that as the level of seashell formulations added to the soil increased, the RNP value increased. This situation would be beneficial for crops (Anderson et al., 2013). With respect to pH, values ranged from 5.8 to 7.2; pH values close to neutral are necessary for the optimal growth of vegetable and crop plants that could be affected by soil acidity (Anderson et al., 2013). The amounts of seashell formulations used were adjusted, and thus the pH values obtained were close to or slightly above 7.0. Ab Manan et al. (2018) reported a similar procedure for testing seashells of commercial mollusks (*Anadara granosa*); the authors required similar amounts of the marine $CaCO_3$ (from 10 to 50 %) added on 10 g soil to achieve pH values (6.52 to 7.16) close to those obtained in the present study.

The *W-OM* formulation (Fig. 1A) allowed the incorporation of 23 g mussel seashells per 100 g of final mass (mussel seashells + soil) to reach values close to 7.0 and a high RNP (93.7%). In the case of *Wo-OM* formulation (Fig. 1B) after adding 19 g of mussel seashells in 100 g of final mass, the achieved pH was close to neutral (7.02), while the RNP was higher than 80.2%. Considering the mixture of equal mass of *W-OM* and *Wo-OM*, 21 g per 100 g of final mass were needed (Fig. 1C) to reach pH values ~7.06 and RNP close to 82.8%. In this sense, Fernández-Calviño et al. (2017) reported that approximately 24 g of a marine $CaCO_3$ were necessary to obtain soil pH values close to neutral (> 6.5), which was consistent with the findings obtained in the present study. Additionally, Fig. 1C shows a zone with high RNP but at a final soil pH below 6.4. This finding could be related to protein-mineral interactions between the organic and non-organic parts of the *50:50* formulation, which decrease the release of calcium from the soil sample matrix (Andersen et al., 2016), thus inhibiting a pH-regulating effect.

The relationship between mussel seashell formulations and soil pH showed that RNP for the alluvial soil varied according to the different pH regulation or buffering capacities of the formulations studied. For each formulation, as the doses applied to the soil increased, pronounced changes in soil pH were observed; however, this effect became less significant with higher seashells levels. The *W-OM* and *50:50* formulations proved to be the most effective for achieving a soil pH close to neutral and high RNP, meaning that a low addition of the formulation was sufficient. The increase in soil pH due to the addition of mussel seashells, could be explained by the neutralization of free protons (H^+ ions) and the constraint of their displacement once they became bound to organic constituents and calcium minerals (Egerić et al., 2019).

The $CaCO_3$ content in the *W-OM* and *Wo-OM* formulations was 82.1 ± 1.9% (w/w) and 92.3 ± 4.8% (w/w), respectively. Even though organic matter in mussel seashells may have accounted for their higher demand to achieve neutral pH, their presence would be positive for increasing soil fertility and availability of nutrients needed by plants (Ahmad et al., 2014). Therefore, the *50:50* formulation could be recommended for pH neutralization of acidic soils and enhancement of nutrient availability to the crops.

A second-order polynomial regression model

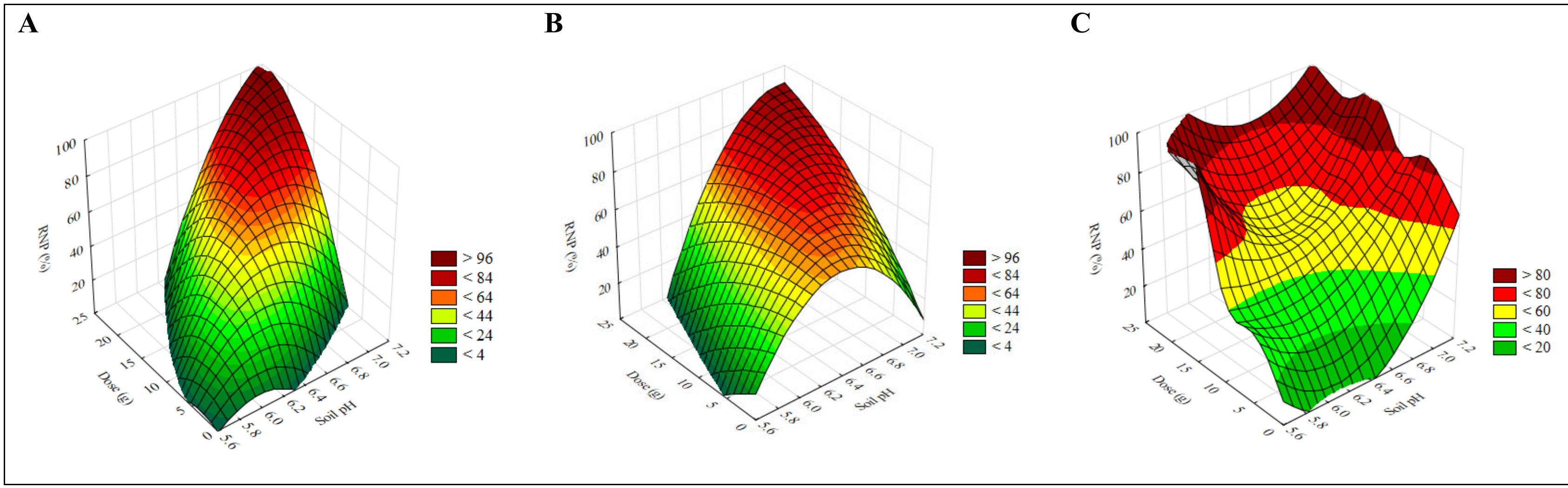

Fig. 1. Response surface analysis of relative neutralizing power (RNP) against the mussel seashell dose incorporated on the acid alluvial soil, and the soil pH resulting from the mixture "mussel seashells + soil". The mussel seashell formulations are presented as: (A) with organic matter or *W-OM* formulation, (B) without organic matter or *Wo-OM* formulation, and (C) a mixture in equal mass ratio of *W-OM* and *Wo-OM* or *50:50* formulations.

was fitted to the experimental results; a significant ($p < 0.05$) second-order effect of soil pH (variable X_2) for *W-OM* formulation, and also a significant ($p < 0.05$) interaction effect between variable X_2 and seashells doses (variable X_1) for *Wo-OM* formulation, were observed (Table 1). The regression models explained more than 94% of the experimental variability (adjusted determination coefficient), which was highly representative. The "best fit" polynomial was observed in the *W-OM* formulation: adjusted determination coefficient (adjusted R^2), > 0.98; mean squared error (MSE), ~ 22; and mean absolute error (MAE), ~ 3.8. For the *W-OM* formulation, the predicted R^2 is in reasonable agreement with the adjusted R^2 because the difference was approximately less than 0.2 units. Additionally, the residual plots show the difference between the actual and fitted observations; a value closer to zero is better, which was observed in the *W-OM* formulation (Fig. 2).

In summary, the results of this study provide valuable information on the use of mussel seashell waste as a source of calcium carbonate for agricultural use. Mussel seashells exhibited a high RNP, while applications in proper doses

Table 1. Regression coefficients of the second-order polynomials that predict the relative neutralizing power of mussel seashell formulations in an acid alluvial soil.

Regression	*W-OM*		*Wo-OM*		*50:50*	
coefficients	Coef.	p-value	Coef.	p-value	Coef.	p-value
β_{12}	0.57	0.0637	0.22	0.0413*	0.44	0.0598
β_{22}	0.04	0.0048*	1.04	0.1929	0.37	0.0507
Predicted R^2	0.9777	-	0.8771	-	0.9337	-
Adjusted R^2	0.9893	-	0.9410	-	0.9682	-
MSE	22.13	-	56.47		47.54	-
MAE	3.81	-	5.19		5.37	-

Where: *W-OM*, was the powder of mussel seashells with organic matter; *Wo-OM*, was the powder of mussel seashells without organic matter; *50:50*, was the powder of mussel seashells mixture in equal parts; β_{12}, β_{22}, are regression coefficients; *, significant differences exist at $p < 0.05$; R^2, is determination coefficient; MSE, is mean squared error; MAE, is mean absolute error.

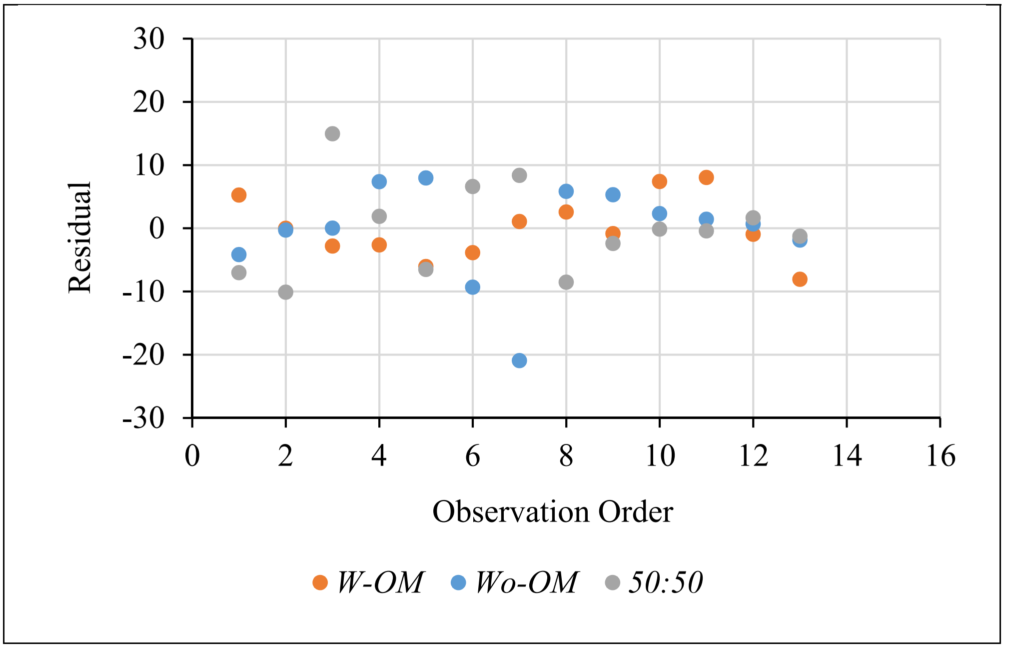

Fig. 2. Residual plot for the second-order polynomial models.

could effectively neutralize acidity and improve the agricultural quality of acidic alluvial soils of Ñuble Coast (Chile).

CONCLUSIONS

Mussel seashells, commonly referred to as waste material on the coasts of southern Chile, can be used as a neutralizing agent in acid alluvial soils of the Ñuble Coast. The response surface methodology allowed modeling the processing conditions to achieve the highest relative neutralizing power of different seashell formulations, highlighting that at least 19 g mussel seashells per 100 g of soil mass were required to achieve a soil pH close to 7.0. Of the three formulations, the *W-OM* formulation exhibited the highest relative neutralizing power (~ 94%). The findings of this work suggest that mussel seashell waste can be potentially used to reduce the use of inorganic fertilizers; as calcium supplement for egg production in hens; to lower ignition point and reduce the probability of soil fires; and/or as natural filters for wastewater cleaning.

ACKNOWLEDGEMENTS

Javier Leiva-Vega acknowledges the support of CORFO-Chile through Proyecto Crea y Valida 21CVS-200234.

DECLARATION OF CONFLICTS OF INTEREST

The authors declare no conflicts of interest.

LITERATURE CITED

Ab Manan, W.N.A., and N.A. Ab Aziz. 2018. Optimization of soil pH by using calcium carbonate ($CaCO_3$) obtained from seashell waste. Gading Journal of Science and Technology 1(1):81-86. https://ir.uitm.edu.my/id/eprint/31354/1/31354.pdf

Ahmad, M., S.S. Lee, J.E. Lim, S.E. Lee, J.S. Cho, D.H. Moon, Y. Hashimoto, and Y.S. Ok. 2014. Speciation and phytoavailability of lead and antimony in a small arms range soil amended with mussel shell, cow bone and biochar: EXAFS spectroscopy and chemical extractions. Chemosphere 95:433-441. https://doi.org/10.1016/j.chemosphere.2013.09.077

Andersen, A., P.N. Reardon, S.S. Chacon, N.P. Qafoku, N.M. Washton, and M. Kleber. 2016. Protein–mineral interactions: Molecular dynamics simulations capture importance of variations in mineral surface composition and structure. Langmuir 32(24):6194-6209. https://doi.org/10.1021/acs.langmuir.6b01198

Anderson, N.P., J.M. Hart, D.M. Sullivan, D.A. Horneck, G.J. Pirelli, and N.W. Christensen. 2013. Applying lime to raise soil pH for crop production (Western Oregon). Accessed on February 20, 2023: https://catalog.extension.oregonstate.edu/em9057

Bünemann, E.K., G. Bongiorno, Z. Bai, R.E. Creamer, G. de Deyn, R. de Goede, L. Fleskens, V. Geissen, T.W. Kuyper, P. Mäder, and M. Pulleman. 2018. Soil quality – A critical review. Soil Biology and Biochemistry 120:105-125. https://doi.org/10.1016/j.soilbio.2018.01.030

Caramantín-Soriano, M. del P., F. Schiappacasse, J. Tapia, P. Peñailillo, and J. Espinoza. 2021. Observations regarding accumulation of metals in wild *Selliera radicans* Cav. in wetland environments of Central Region of Chile. Idesia (Arica) 39(3):5-11. https://dx.doi.org/10.4067/S0718-34292021000300005

Egerić, M., I. Smičiklas, B. Dojčinović, B. Sikirić, M. Jović, M. Šljivić-Ivanović, and D. Čakmak. 2019. Interactions of acidic soil near copper mining and smelting complex and waste-derived alkaline additives. Geoderma 352:241-250. https://doi.org/10.1016/j.geoderma.2019.06.015

Fernández-Calviño, D., L. Cutillas-Barreiro, A. Núñez-Delgado, M.J. Fernández-Sanjurjo, E. Álvarez-Rodriguez, J.C. Nóvoa-Muñoz, and M. Arias-Estévez. 2017. Cu immobilization and *Lolium perenne* development in an acid vineyard soil amended with crushed mussel shell. Land Degradation & Development 28(2):762-772. https://doi.org/10.1002/ldr.2634

Fu, H., X. Jian, W. Zhang, and F. Shang. 2020. A comparative study of methods for determining carbonate content in marine and terrestrial sediments. Marine and Petroleum Geology 16. https://doi.org/10.1016/j.marpetgeo.2020.104337

Goulding, K.W.T. 2016. Soil acidification and the importance of liming agricultural soils with particular reference to the United Kingdom. Soil Use and Management 32(3):390-399. https://doi.org/10.1111/sum.12270

Hirzel, J. 2020. Suelos de la Región de Ñuble: Caracterización general [en línea]. Chillán: Colección Libros INIA – Instituto de Investigaciones Agropecuarias. N°39. Available in: https://hdl.handle.net/20.500.14001/3626 (Consultado: 5 Julio 2023).

Hirzel, J., R. Lecannelier, E. Cuevas, and F. Rodríguez. 2017. Efectividad de cales granuladas en el manejo de dos suelos ácidos. Archivo Agronómico 16:21. https://fertilizar.org.ar/wp-content/uploads/2021/09/21.pdf

Lu, H., Y. Wu, P. Liang, Q. Song, H. Zhang, J. Wu, W. Wu, X. Liu, and C. Dong. 2020. Alkaline amendments improve the health of soils degraded by metal contamination and acidification: Crop performance and soil bacterial community responses. Chemosphere 257. https://doi.org/10.1016/j.chemosphere.2020.127309.

Muñoz-Sepúlveda, J.I. 2020. Análisis hidrogeoquímico y diagnóstico de los recursos hídricos en la comuna de Ninhue, Región del Ñuble, Chile (Doctoral dissertation, Universidad Andrés Bello). Accessed on February 20, 2023: http://repositorio.unab.cl/xmlui/handle/ria/18592

Saleh, I., S. Syamsir, V. Pramaningsih, and H. Hansen. 2021. The use of green mussel as bioindicator of heavy metal pollution in Indonesia: A review. Environmental Analysis Health and Toxicology 36(4):e2021026. https://doi.org/10.5620/eaht.2021026

Singh, J., and A.S. Kalamdhad. 2011. Effects of heavy metals on soil, plants, human health and aquatic life. International Journal of Research in Chemistry and Environment 1(2):15-21. ISSN 2248-9649.

Summa, D., M. Lanzoni, G. Castaldelli, E.A. Fano, and E. Tamburini. 2022. Trends and opportunities of bivalve shells' waste valorization in a prospect of circular blue bioeconomy. Resources 11(5): 48. https://doi.org/10.3390/resources11050048

Ulloa-Jara, R.A. 2022. Análisis hidrogeológico de dos sistemas de vertientes pertenecientes al valle del Renegado, Región de Ñuble, Chile. Accessed on February 20, 2023: http://repositorio.udec.cl/bitstream/11594/10488/1/Tesis%20Rodrigo%20Ulloa.pdf

Zhan, J., J. Lu, and D. Wang. 2022. Review of shell waste reutilization to promote sustainable shellfish aquaculture. Reviews in Aquaculture 14(1):477-488. https://doi.org/10.1111/raq.12610