Quality Improvement of Eutrophic Environments Degraded by Organic Matter, in Experiences Conducted in Sea-Water Microcosms

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ABSTRACT

An experience in seawater microcosms was conducted to evaluate the effectiveness of the zeolite chabazite (CHA) in the mitigation of water eutrophication produced by sediments with high organic load. The experience was conducted in microcosms with addition and in the absence of effective microorganisms (EM). In the absence of EM, the ammonium abatement by CHA compared to the controls without CHA, was elevated only in the first week, while with the addition of EM, the abatement occurred for the entire experience (one month), although gradually reducing, as ammonia releases increased over time. Ammonium releases were 1.4 to 2.3 times lower in CHA microcosms compared to CHA-free controls and, among those in which CHA was present, they were lower in the presence of EM. Soluble orthophosphates also showed a reduction in microcosms with CHA, compared to the control microcosms, with a more marked results in the absence of EM, probably due to the formation of insoluble salts.

Keywords: Ammonium, chabazite, eutrophication, microcosms, phosphorus, zeolite.

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I. INTRODUCTION

Zeolites, treated or natural, made up of aluminosilicates with tectosilicate scaffolding, with sodium (Na^+), calcium (Ca^{++}), magnesium (Mg^{++}) e potassium (K^+) ions, can subtract ammonium ions (NH_4^+) from the water, having the ability to carry out cationic exchanges.

Many studies have been done on zeolites. Studies in which zeolites were used as ion exchangers to absorb ammonia, focused on agricultural use (Passaglia, 2008; Comba *et al.,* 2012; Faccini *et al.,* 2015; Colombani *et al.,* 2015) others revealed positive effects on fresh water (Briggs & Funge-Smith, 1996) and tested the potential purification of zeolite in waterworks and sewage disposal for the reduction of ammonia and heavy metals (Markoska & Reka, 2013; Kumar et al., 2021; Muscarella *et al.,* 2021) and for wastewater treatment in small freshwater aquaculture plants (Ciambelli *et al.,* 1984; Berger *et al.,* 1991) and more recently also in seawater's sea-bass juvenile breeding test (Aly *et al.,* 2016).

Due to the high presence of cations competing with ammonia the purification of high salinity water was not considered feasible with zeolite (Chiayvareesajja *et al.,* 1993). Furthermore, the ion exchange capacity of zeolite in marine waters should be reduced due to the abundant presence of dissolved organic matter (Wheaton, 1982). However, López-Ruiz & Goméz-Garrudo, (1994) conducted an experience in sea water showing that, compared to the fresh-water, the ammonium subtraction is possible if a greater amount of zeolite is used.

The impact of aquaculture is becoming progressively more and more important: in the last 35 years, the production of marine aquaculture has grown from 10 million tones to about 55 million tones (FAO, 2006). Land-based and nearshore fish farms have critical aspects in the impact of wastewaters on marine coastal areas. Wastewaters are rich in dissolved nutrients, with high concentrations of ammonium ion (Porrello *et al.,* 2003a). Modest results can be obtained with treatment ponds based on purification by macroalgae (Porrello *et al.,* 2003b; Gennaro *et al.,* 2005) and the impact still remains important. The eutrophicating wastewaters in aquacultures can induce algal growth in the final receiving basin and in the breeding ponds, determining the accumulation of labile organic matter in the sediments and favoring dystrophic phenomena with releases of ammonium, orthophosphates, and hydrogen sulfide from the sediments, and eventually the die-offs of the fish. In this regard, the use of zeolite for ammonium subtraction could result in a mitigation solution for the wastewater emitted from these production activities.

The purpose of this study was to test the action capacity of the chabazite zeolite against ammonium and orthophosphate ions in seawater on sediments with a high organic load, with the addition (or not) to the microcosms of effective microorganisms (EM) to regenerate the chelating sites of the zeolite, once saturated with ammonium.

II. MATERIALS AND METHODS

A. Zeolite

There are different types of zeolites, with different ratios between silicon and aluminum and iron (Si:(Al + Fe)): clinoptilolite and mordenite have a high ratio, heulandite, chabazite, phillipsite and erionite have intermediate ratio values, analcime and laumontite have low ratio values. The preference of cations for the various zeolites normally follows the sequence $K^+>NH_4^+>Na^+>Ca^{++}>Mg^{++}$, therefore the replacement of the metal cation with NH_4+ depends very much on the ionic concentration of NH_4^+ itself and of the competing cations (Colella, 1996).

Most studies that demonstrated the ammonia removal efficiency of zeolites have been conducted using clinoptilolite (CPL), while very few studies have looked at the performance of other natural zeolites. On the other hand, CPL is one of the most abundant natural zeolites and it showed ammonia adsorption capacities ranging from 3.42-9.12 mg NH_4^+ g-1 CPL (Murphy *et al.,* 1994). The effectiveness of ion absorption also depends on the size of the pores present in the zeolite. The intracrystalline cavities can vary for the various zeolites, between 20% and 50% of the total volume of the crystalline body. CPL has a pore volume of 20%, chabazite (CHA) of 30% (Flanigen, 1983).

The zeolite adopted in this experience was CHA, obtained from the processing residues of a quarry sited in Sorano (South Tuscany, Italy). This zeolite contains mainly K^+ in the pores. NH_4^+ ions are subtracted from the crystal structure of CHA and are chelated within the crystal structure by replacing the K^+ ions. The subtraction of NH_4^+ consequently leads to a reduction of undissociated ammonia (NH_3) which is in equilibrium with its ion in a chemical equilibrium ratio dependent on pH, T and salinity (Amend *et al.,* 1982). However, there is a limit to the amount of NH_4^+ that can be subtracted from the aqueous solution, which depends on the availability of ion substitution pores, therefore the mineral is subjected to saturation.

Zeolite can be regenerated using a solution with a concentration of cations (Na^+ for CPL and K^+ for CHA) higher than that of NH_4^+ (Wheaton, 1982). But this is not a viable method in relatively large environments such as reservoir beds or canals where wastewaters flow from the production activities. The best regeneration in these cases, is the biological one, i.e., favoring the bacterial processes of nitrification and denitrification. Bacteria are added to the treatment, attaching themselves to the zeolite crystals. When a large load of NH_4^+ flows into the system, the bacteria begin to convert the NH_4^+, but they are unlikely to be able to transform the entire load. Zeolites will absorb the NH_4^+ overdose and when NH_4^+ concentration decreases, the chelated NH_4^+ is released into the water and converted by bacteria.

The nitrification process always requires oxygen and the introduction of air directly into the system is encouraged. However, in open systems, such as small shallow basins or canals, a modest oxygenation can occur sufficiently with the water movement produced by the wind and, in any case, it can be easily increased with the oxygenation tools used in aquaculture. An inevitable negative consequence is the formation of bacterial biofilms that covers the zeolite, which could reduce the ion exchange capacity by up to 30% (Lahav & Green, 1998).

B. Effective Microorganisms

To make the process repetitive, zeolite can be enriched with nitrifying/denitrifying bacteria and other bacterial strains that break down organic matter. Nitrifying bacteria oxidize NH_4^+ to nitrate (NO_3^-), while denitrifying agents act on the latter by eliminating it from the system as N_2O and N_2 gaseous. New NH_4^+ can then be trapped in the crystal structure. These bacterial strains are found in the natural environment and could naturally form films around the zeolite crystals. However, artificial enrichment can improve the process.

The effective microorganism activated solution (EMAS) technology was developed in the early 1980s (Higa & Parr, 1994). The EM solution we used is a blend of 88 microbial strains (lactobacillus spp., yeast, photo-synthetic bacteria, nitrifying bacteria and denitrifying bacteria) marketed by EMRO Japan (https://emrojapan.com/aquaculture/). The mixture should favor and accelerate the decomposition and mineralization processes of organic matter by improving the quality of eutrophic environments degraded by organic matter (Zakaria *et al.,* 2010).

C. Operating Procedure

The main objective of this experience was to evaluate the behavior of the mineral in pre-dystrophic/dystrophic conditions, in order to use zeolite for the mitigation of highly critical eutrophic environments.

To this end, two experimental tests were conducted in June-July 2021. Both tests were aimed at the quantitative analytical determination of ammonia nitrogen (N- NH_4^+) and reactive soluble phosphorus (SRP; orthophosphate ion, PO_4^{3-}) and at a qualitative evaluation of H_2S.

In a first set of 6 glass cylinders, 2 L of volume each, microcosms were produced with sea water (38 psu) and muddy lagoon sediment enriched with labile organic matter (LOM). A pair of cylinders (A, B) constituted the controls with respect to the treatments. The second pair (C, D) and third pair (E, F) were added with a layer of CHA, with fine grain size (1-3 mm in diameter) and coarse grain size (3-10 mm), respectively. In this set of microcosms, CHA was washed to remove dust and subsequently was enriched with 5 mL of the solution containing EM.

The second group consisted of 6 glass cylinders, 2 L of volume each, filled with sea water and lagoon sediment enriched with LOM. Three cylinders (G, H, I) were used as a control, free of zeolite, and three cylinders (J, K, L) were used as a treatment with the addition of a surface bed of coarse zeolite (3-10 mm of diameter), washed to remove dust, without adding EM.

The last two cylinders (M, N) were filled with seawater and lagoon sediment, without adding LOM and zeolite.

For all cylinders, the wet lagoon sediment was sieved to 1 mm and placed in a beaker where it was homogenized. For the group A-F, the sediment was subsequently mixed with industrial hummus and crude protein. For the G-L group, the sediment was enriched with hummus only to reduce the amount of ammonium released by the decomposition processes in the absence of an additional bacterial pool. For the M-N group, no additions were made.

Each cylinder received about 310 g of mud collected in the

Orbetello lagoon (Tuscany, Italy), equal to a volume of 92 mL and a thickness of 6 cm of the cylinder. The enrichment in carbohydrates, proteins and lipids for the A-L group is shown in Table I.

Cylinders of the C-D pair received an over-layer of fine zeolite+EM of about 1.5 cm (21 mL, 47 g), the E-F pair received an over-layer of coarse zeolite+EM of about 2 cm (28 mL, 46 g) and those of the J-L group received a 1.5 cm coarse zeolite over-layer (20 mL, 34 g) (Table I). Subsequently, 1500 mL of previously pasteurized sea water was added to each cylinder, in a light drop-by-drop flow to avoid disturbance in the sediment layers. The experience began on June 27, 2021 and ended on July 27, 2021. In each cylinder 500 mL of water was taken for analysis after one week, two weeks and one month from the starting time of the test. Samplings were conducted by siphoning without stirring the microcosms and the waters were replaced for the same volume with the original pasteurized sea water in light flow to avoid affecting the sediment layer. During the experience, quantities of distilled water were added to counteract evaporation. The experience was conducted in a laboratory environment sheltered from sunlight.

The water samples for analysis were refrigerated and sent to the laboratory in 2 hours from the collection.

For all samples, N-NH4 and SRP were determined. Samples were filtered at 0.45μm and the following analytical determinations were conducted according to APAT IRSA-CNR, (2003). The atomic ratio N-NH4: SRP was then computed.

The possible subtraction/enrichment of each nutritional component in the water column was calculated by comparison of each data obtained from the cylinders with zeolite (treatments) with each data of the cylinders without zeolite (controls), according to (1):

$$(t - c) \times 100 \times c^{-1} \qquad (1)$$

where c is the concentration of a given chemical species of one of the controls, and t is the concentration of the same chemical species of one of the treatments. A negative result expresses decrease, while a positive result an increase.

At the start of the test, sediments, both the original ones and the enriched ones, were subjected to analysis of the percentage of labile organic matter (LOM) as loss on ignition at 250 °C for 6 h after drying at 75°C for 48 h (Loh, 2005). Determination was not conducted at the end of the experience since the result would have been altered by the presence of zeolite debris.

The presence of acid-volatile sulfides (AVS = H_2S + HS^- + S^{2-}) was highlighted every day for all microenvironments after sampling 1 mL of solution to which a drop of ferrous chloride solution ($FeCl_2$) was added: the presence of AVS was highlighted by the formation of a black colored precipitate due to the formation of FeS.

Dissolved oxygen (DO; mg L^{-1} and as percentage of saturation; by OxyGuard Handy Mk III oxymeter), pH (pH scale; by DELTA OHM HD8705) and temperature (T °C; by electronic digital thermometer) were measured. DO and pH were measured every 72 hours, T every 12 hours.

Results of the water nutrient of the microcosms A-N are reported in Table II. In Table III, for the groups A-F and G-L, the mean (± SD) increases (+) and decreases (-) of the two nutrient components examined are reported. The averages were obtained between each record of C-D and E-F cylinder samples and each value obtained from the respective A-B control cylinder samples, and, similarly, between the records of JKL cylinder samples and each value of GHI control cylinder samples.

TABLE I: EXPERIMENTAL CONDITIONS OF MICROCOSMS A-N. CHA, CHABAZITE; CHA-1-3 G, CHA-3-10 G, GRAMS OF CHA INSERTED IN THE CYLINDERS, WITH GRAIN SIZE OF 1-3 MM E 3-10 MM

Cylinders	A	B	C	D	E	F	G	H	I	J	K	L	M	N
Volume L	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5	1.5
Sed cm	6	6	6	6	6	6	6	6	6	6	6	6	6	6
Sed g	310	310	310	310	310	310	310	310	310	310	310	310	310	310
Proteins mg	401	401	401	401	401	401	79	79	79	79	79	79		
Carbohidr. mg	290	290	290	290	290	290	290	290	290	290	290	290		
Lipids g	149	149	149	149	149	149	13	13	13	13	13	13		
CHA 1-3, g			35	35										
CHA-3-10, g					46	46								
CHA 3-10, g										34	34	34		
T°C	28-32	28-32	28-32	28-32	28-32	28-32	28-32	28-32	28-32	28-32	28-32	28-32	28-32	28-32

TABLE II: VALUES IN μM OF N-NH4 AND SRP AND THEIR MOLAR RATIO NH4 : SRP ESTIMATED IN THE THREE WATER SAMPLES CONDUCTED AFTER ONE WEEK (7D), TWO WEEKS (14D) AND ONE MONTH (30D), ON CYLINDERS A, B (CONTROLS WITH ENRICHED SEDIMENT), C, D (TREATED WITH FINE ZEOLITE, WITH ENRICHED SEDIMENT), E, F (TREATED WITH COARSE ZEOLITE, WITH ENRICHED SEDIMENT), G, H, I (CONTROLS WITH ENRICHED SEDIMENT), J, K, L (TREATED WITH COARSE ZEOLITE, WITH ENRICHED SEDIMENT), M, N (CONTROLS WITH NON-ENRICHED SEDIMENT).

		7D			14D			30D		
		N-NH4	SRP	NH4: SRP	N-NH4	SRP	NH4: SRP	N-NH4	SRP	NH4: SRP
		μM	μM		μM	μM		μM	μM	
	A	4328	16.419	264	5503	17.710	311	5412	44.323	122
	B	3638	8.806	413	4830	5.323	907	5520	32.323	171
	C	1338	8.677	154	1922	1.065	1806	3998	38.968	103
	D	850	8,29	103	1204	1.000	1204	3463	9.774	354
	E	219	8.226	27	697	0.032	21600	2765	23.484	118
	F	612	9.226	66	1457	0.839	1737	3544	18.452	192
	G	705	1.387	508	1062	2.419	439	2388	5.161	463
	H	763	2.097	364	1391	3.806	365	1740	5.065	344
	I	1201	2.613	460	1759	4.323	407	2310	6.387	362
	J	254	0.839	303	1091	1.613	676	1551	2.516	616
	K	149	0.290	513	822	1.871	439	1346	2.355	572
	L	283	0.355	798	1539	2.194	702	1982	3.903	508
	M	0.07*	0.160*	0.44*	360	2.129	169	320	1.813	177
	N	0.07*	0.160*	0.44*	267	1.900	141	221	1.060	208

* Values refer to the sea-water used initially.

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In the A-F cylinders, gas bubbles were observed 24 hours from the start of the test, but without AVS presence. After 5 days, the first formation of AVS was highlighted, and the cylinder A showed the greatest intensity of the color in the $FeCl_2$ test. At 24 hours from the dilution following the second sampling, all A-F cylinders showed a high presence of AVS. A similar process occurred for the G-L set, with the G, H, I group showing the greatest intensity of black coloring in the ferrous chloride test from the first week, while the treated group showed the same intensity but only starting from the second week. In the microcosms M, N no gas formations were observed and the $FeCl_2$ test did not show AVS production.

The twelve microcosms A-L quickly reached anoxia condition and in the first week, in the middle of each cylinder, DO values varied between 0.34 and 0.25 mg L^{-1} (4.7-4.0% saturation). pH was on average 8.2±0.2 and 7.87±0.1, for the control cylinders and CHA cylinders, respectively. For all cylinders, temperature was maintained between 28 and 32 °C during the whole experience. CHA zeolite seems to slightly acidify the water column by an average of 0.33 pH units.

With regard to the LOM sediment content, the lagoon mud filtered at 1 mm was found to have a labile organic matter content of 7.11±0.32% (including M-N control cylinders),

while the addition of hummus and hummus + proteins resulted in a value of 7.67±0.37% and 7.70±0.46%, respectively.

$N-NH_4^+$ concentration increased in all cylinders during the experience, due to the anaerobic demolition of labile organic matter. The effects of the zeolite on ammonium releases from the sediment were very important at the end of the first week, in both the C-F and J-L groups. In the absence of EM, the ammonium abatements compared to controls were drastically reduced in the samples following the first week, showing inhomogeneous behaviors. With the addition of EM, the abatements, although tending to flex compared to the first 7 days, continued to be imported and homogeneous among the samples with greater efficacy for coarse zeolite than for finer texture (Table III).

In the fine-grained zeolite cylinders (C-D), the percentage of $N-NH_4^+$ abatement, compared to the control, was 72% after one week, 70% after two weeks and 32% after one month. In the cylinders with coarser grain zeolite (E-F), $N-NH_4^+$ was lower than the control ones for 89%, 79% and 42% for the same sampling periods (Table III).

SRP concentration also increased over time, in both tests and in the controls with non-enriched sediment, with the exception of the second week for the C F cylinders, due to a drastic reduction compared to the two controls A, B and compared to the concentrations of the first week (Table II).

In the EM test, SRP abatements were effective only in the second week for the C-D cylinders and in the second and fourth weeks for the cylinders E-F. In the test in the absence of EM, SRP was significantly lowered, in the first week, in JKL compared to the controls GHI, and the abatement remained relatively high in the following weeks as well (Table III).

TABLE III: AVERAGE (± SD) BETWEEN THE ESTIMATED REDUCTION/INCREASE OF AMMONIA NITROGEN (N-NH4) AND OF ORTHO-PHOSPHATE (SRP), OBTAINED BETWEEN EACH RESULT OF CYLINDERS C-D AND E-F, WITH RESPECT TO THE VALUES OF CONTROL CYLINDERS A-B, AND OF THE CYLINDERS JKL VERSUS THE CONTROL CYLINDERS GHI, FOR THE SAMPLES CONDUCTED AFTER ONE WEEK (7D), TWO WEEKS (14D) AND ONE MONTH (30D) FROM THE START OF THE EXPERIENCE

	7D		14D		30D	
	N-NH4	SRP	N-NH4	SRP	N-NH4	SRP
CD v AB	**-72.32±6.64**	-26.00±22.40	**-69.61±7.27**	**-87.39±6.80**	**-31.75±4.94**	-34.81±40.82
EF v AB	**-89.48±5.07**	-23.89±23.42	**-79.07±7.52**	**-94.68±6.28**	**-42.29±7.15**	**-43.91±11.11**
GHI v JKL	**-72.82±9.6**	**-73.94±16.02**	-14.47±30.29	**-42.76±17.79**	-22.67±18.18	**-46.60±14.69**

In bold, the most consistent and low-variability nutrient abatements.

In the control cylinders M-N, $N-NH_4^+$ and SRP increases were observed compared to the values of the starting water, meaning that the sediment released nutrient in anaerobic conditions based on the organic matter quantity already present in the starting lagoon sediment. However, $N-NH_4^+$ and SRP releases estimated in M-N in the second week, were respectively 17 and 5.7 times lower than the releases of the controls A and B, enriched with carbohydrates and proteins, and respectively 4.5 and 1.8 times lower than those released from the GHI controls, mainly enriched with carbohydrates.

Furthermore, we considered the amount of ammonia nitrogen removed with the three samples conducted in each microcosm (1500 mL) and the amount left in the residual water of each microcosm (1000 mL). Applying (2) and (3), these quantities were reported to the corresponding mg of proteins, the results of which are reported in Table IV as means for the control groups A-B, GHI and M-N as well, and for the treatments C-D, E-F and JKL. In this table, it can be

observed that the amounts of equivalent protein released by the C-D and E-F treatments were 1.8 and 2.3 times lower than the AB controls, respectively, and about 1.4 times lower than in the GHI controls for the JKL treatments. The M-N controls, whose sediments were devoid of organic enrichment, released 20- and 7-times lower amounts of equivalent proteins than the A-B and GHI controls, respectively.

$$\mu M \times 14 \times 1000^{-1} \times 2^{-1} = mg\ N\text{-}NH_4 \qquad (2)$$

where mM is the concentration of ammonia nitrogen expressed in micromoles per liter, 14 is the molecular weight of N, 1000^{-1} the factor to convert micro- in milli-, 2^{-1} the factor to convert the ammonium quantity in 1000 mL to the quantity in 500 mL.

$$mg\ N\text{-}NH_4 \times 100 \times 16^{-1} = mg\ equivalent\ proteins \qquad (3)$$

where 100×16^{-1} are the factors to bring the amount from the estimated ammonia nitrogen to the amount of corresponding proteins.

N-NH$_4$: SRP ratio was always very high in all microcosms, between 66 and 21600, excluding the only low value of 27. The median was 333, 689, 349 for the first, second and fourth week, respectively. The rise was also observed in microcosms M-N with non-enriched sediment. In the G-L group, N-NH$_4$: SRP ratio tended to increase in the cylinders treated with zeolite compared to the controls, especially in the second and fourth week. On the contrary, for C-D and E-F groups, in the first week the molar ratios were reduced, while subsequently the behaviors were variable and inconsistent with each other.

TABLE IV: RP, MEAN (± SD) OF THE RELEASED PROTEINS, CORRESPONDING TO THE QUANTITY OF AMMONIA NITROGEN REMOVED FROM THE MICROCOSM SYSTEM DURING THREE 500 mL SAMPLINGS AND TO THE FINAL RESIDUE OF 1000 mL PER MICROCOSM, FOR A-B, GHI AND MN CONTROLS, FOR C-D, E-F AND JKL TREATMENTS. AP, PROTEINS INITIALLY ADDED TO THE SEDIMENT. RP/AP, RELEASED PROTEINS AND ADDED PROTEINS RATIO

	RP	AP	RP/AP
	mg		
A, B	1117±23	401	2.8
C, D	606±61	401	1.5
E, F	479±76	401	1.2
G, H, I	382±45	79	4.8
J, K, L	274±50	79	3.5
M, N	56±9		

III. DISCUSSION

The effects of zeolite on ammonium releases from the sediment in the microcosm tests, were observed to be very effective especially in the first week, meanwhile, in the absence of EM, the effects were subsequently or drastically reduced. After the first week, the reduction in the percentage of abatement with respect to controls, both in the microcosms with the addition of EM, and, above all, in those without any bacterial reinforcement, was likely due to a progressive saturation of the active sites of the zeolite, and to a production of ammonium higher than the nitrification/denitrification efficacy of the available natural bacterial pool (Table II). On the other hand, ammonium gradually increased throughout all the examined periods. In fact, in controls A and B, the ammonium concentration went from 3983±345 mM to 5166±366 mM, and to 5466±54 mM, for the first, second and fourth week, respectively. Similarly, for GHI controls, ammonium went from 889±222 mM to 1404±285 mM and to 2146±289 mM, in the same temporal order of sampling.

SRP behavior was less consistent between the two groups C-F and JKL, in fact its releases in the water column were more efficiently countered in the JKL group. It seems unlikely that the reduction of orthophosphates can be attributed to a direct action of the zeolite, both in terms of chelating capacity for an ion too bulky such as orthophosphate, and for the slightly acidifying action on the water column which showed a lowering of about 0.33 pH units in the water column, compared to microcosms without CHA. It is more credible that an increase in pH and/or Ca^{2+} ions may have occurred, at least in the sedimentary interstices and in the water column underlayer close to the sediment, which favored the precipitation of orthophosphates. The

undissociated ammonia produced by bacterial activity may have contributed to an increase in pH in interstitial waters, the effect of which may have been different in the various microcosms in relation to the amount of hydrogen sulfide produced, which may have counteracted or favored the alkaline effect. The same action of the sulphate-reduction bacteria is often an alkalizing action, although it can be acidic or alkaline depending on the electron donor, or the quality of organic matter that provides energy to the bacteria (Meulepas *et al.,* 2010). All these factors may have determined a further condition of variability among the microcosms.

We examined N-NH$_4$: SRP molar ratio since the ammonium ion is the dominant species in the anaerobic conditions where we placed the microcosms. The values were always very high to underline the abundance of ammonia releases, normally higher than those of dissolved orthophosphate. This was also observed in the M-N microcosms, where, starting (like all the other microcosms) from the water where ammonium was almost absent and therefore from an N-NH$_4$: SRP ratio close to zero, even in a moderate anaerobic condition, ammonium release prevailed over orthophosphate.

The results argue that the use of EM may have prolonged the effects of ammonia reduction, albeit with gradually decreasing values. On the other hand, the enrichment in LOM allowed the anaerobic bacterial activity to increase the ammonium concentration as the experience progressed, obviously more in the C-F group than in the J-K-L group, the latter lacking the addition of crude protein.

The enrichment in LOM, albeit modest, around 840 mg and 382 mg overall (carbohydrates + proteins + lipids) for the A-F and G-L groups, respectively (Table I), which recorded in the LOM estimates between original sediment and enriched sediment an average D of 0,56% and 0,59%, was the trigger for an intense bacterial activity that also attacked the organic matter already present in the sediments, producing an intense sulphate-reducing activity. Indeed, H$_2$S developed everywhere, although in microcosms with CHA lagging behind those in which the mineral was absent. This did not happen in the microcosms M-N, in which no enrichment in organic matter was carried out, although the D compared to the enriched sediment had been relatively modest. It is confirmed that to evaluate the dystrophic potential of a sediment it is important to know its LOM, but it would be even more important to know its composition and the protein/carbohydrate ratio (Pusceddu *et al.,* 2009).

The ammonium removed with the samples plus the residual one in the microcosms at the end of the experience, reported as equivalent proteins in Table IV, were overall much greater in A-B than in the treatments, especially compared to E-F. The GHI group also had overall higher ammonium releases than the JKL treatments, although the difference between control and treatment was less than that found in the A-F group. Furthermore, the ratio between the released proteins and those added initially resulted much higher in the GL test compared to AF test, despite the lower amount of protein initially added, as if adding only the carbohydrates in GL stimulated bacterial activity towards the protein pool already present in lagoon sediments. From these results, it can be inferred that ammonia releases were greater in the absence of both CHA and effective microorganisms.

The progressive increase in ammonia concentration, with the progress of the bacterial demolition processes, allows us to hypothesize that the CHA zeolite gradually becomes saturated with nitrogen and/or that the nitrification/denitrification process was slower than the bacterial ammonification process of the organic matter present in the sediment. Therefore, in order to use the zeolite for the reduction of the nutrient load released by fish-farms of marine aquaculture or directly inside ponds and canals, it can be inferred that the effectiveness of CHA over time depends very much on the quantity of labile organic matter present in the sediments (or, from another point of view, the quantity of CHA used), and that the greatest effectiveness of the treatment occurs for those concentrations of LOM that allow an equivalence of times between ammonium production and nitrification/denitrification. Ultimately, an enrichment of CHA with EM or bacterial strain acclimatized to the area in which it operates could accelerate the nitrification/denitrification process and determine a more effective ammonium abatement over time. However, other problems remain to be solved, for example to avoid that the mineral remains submerged by the fall-out of sedimentation, perhaps by acting with periodic reshuffling of the sedimentary surface layer.

IV. CONCLUSION

The use of zeolite chabazite in the treatment of sediments with high organic load, such as those of coastal areas with poor water exchange, fish farming ponds, constructed wetlands of wastewater from land-based fish farms, etc., can produce important mitigation effects.

In this experience, we observed a substantial reduction in the load of ammonia nitrogen and orthophosphates in microcosms treated with chabazite in seawater, compared to controls that did not have any. With the addition of EM, the reduction of the ammonia load compared to the control occurred for the entire month of experience, albeit in very high ammonium concentrations that were progressively increasing. In the absence of EM, the abatement was effective for the first week, subsequently the microcosms with the treatment behaved differently with each other with releases that in some cases exceeded those of the comparison. As a result, the action of the chabazite should be accompanied by an effective denitrifying bacterial activity.

The limit that is highlighted, in this type of treatment of the eutrophication effects, consists precisely in the regeneration of the zeolite active sites, once saturated with ammonium, especially if we operate in environments with a high organic load. In large-scale interventions, it is evident that a chemical treatment cannot be examined or even repeatedly intervene with a new mineral. It is necessary to work on an enrichment with suitable bacterial strains, able to reduce the ammonium concentration allowing the gradual restitution of the chelated ions. Basically, the zeolite would have the function of accumulation lung of the ammonium ion when the pulses releases from the sediment or periodic increases in concentration in the wastewater occur, allowing the bacterial pool to operate on greater quantities of ammonium than would happen in its absence.

This study was set up on an extreme case, such as to produce conditions of high degradation, and therefore of high ammonium releases, in order to test the mineral efficiency. While it is clear that in full-scale conditions it is necessary to appropriately balance the chabazite in relation to the eutrophic dynamics of the intervention site, i.e., to seek as much as possible a balance between the amount of ammonium that can be retained by the mineral, the effectiveness of the nitrifying/denitrifying bacterial pools and the amount of ammonium that can be released in a given condition by the sediment and/or productive activities.

V. CONFLICT OF INTEREST

None of the authors have any conflicts of interest.

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