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Toxicity evolution of alum-coagulated municipal wastewater to sea urchin embryogenesis and fertilization

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ABSTRACT

The alum-based coagulation process is a worldwide treatment method in industrial and municipal wastewater (MW) management. In the present study, alum-coagulation was performed on wastewater from the MW plant located south of Naples, Italy, to evaluate any negative impact of MW on marine biota by means of sea urchin bioassays. A series of Jar test experiments (100 rpm for 1 min, 30 rpm for 20 min, and 30 min for settling) was performed using 150 and 450 mg/L of alum concentrations at pH ranging from 5 to 7 and room temperature (20˚ ± 2˚C). Raw and alum-coagulated wastewater samples were analyzed for their COD and TSS, and residual aluminum (RA) concentrations. Toxicity testing of samples (diluted to 1%) by *Sphaerechinus granularis* sea urchin bioassays (collected in Naples bay) was evaluated to meet the following specifications: (a) acute and/or developmental toxicity, (b) changes in fertilization success, and (c) cytogenetic abnormalities. The results provided the following evidences: (i) the coagulation process at a 150 mg/L alum level in the SG plant was sufficient to meet the discharge standards; (ii) RA was found significantly related to alum dose used in coagulation process and raw wastewater alum concentration; (iii) sea urchin bioassays provided evidence for both embryo toxicity in raw wastewater to a lesser extent, and in alum-coagulated Jar test supernatants, at elevated extent, at a 1% concentration; and (iv) no statistically significant spermiotoxic or cytogenetic effect was observed. Altogether, the results highlighted the influence of wastewater characteristics on developmental toxicity, such as pH-related Al(III) speciation and alum complex formation in wastewater.

Keywords: Toxicity; Coagulation; Alum; Residual aluminum; Municipal wastewater; Sea urchins

1. Introduction

The coagulation–flocculation process in wastewater treatment provides a high removal efficiency of TSS and COD using Al(III) or Fe(IIII) salts, with or without the use of a base (as calcium salts or NaOH). Coagulant dosages vary in a wide range aiming at maximum removal efficiency of pollutants using minimum doses at optimum pH. A wide concentration range of $Al_2(SO_4)_3{\cdot}18\ H_2O$ was used for treatment of domestic (50 to 500 mg/L) and industrial (100 to 1,500 mg/L) wastewaters, respectively [1], and alum

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(20 to 40 mg/L) was applied for drinking water production [2]. Aluminum in coagulated drinking water and in wastewater effluent has been regarded as a subject of human and environmental health concern [3]. Aluminum concentration in natural waters is usually lower than 100 μg/L; however, its concentration and speciation between toxic (free Al^{+3} and Al–OH) and complex (Al–F and Al-Org complexes) forms varied extensively due to seasonal changes and pH of water bodies [4]. A wide range of residual aluminum (RA) (0–530 μg/L) in alum-coagulated tap water was detected in Harris County, Texas [5]. Driscoll et al. [6] reported that the use of alum increased the total Al(III) concentration from 0.37 ± 0.33 μmol/L in raw water to 1.8 ± 0.33 μmol/L in alum-coagulated filtered water. Several approaches are available for minimizing RA concentrations in treated water. These include use of optimum pH in the coagulation process, avoiding excessive aluminum dosage, good mixing at the point of application of the coagulant, optimum paddle speeds for flocculation and efficient filtration of the aluminum floc. Under good operating conditions, concentrations of aluminum of 0.1 mg/L or less are achievable in large water treatment facilities. Small facilities (e.g. those serving fewer than 10,000 people) might experience some difficulties in attaining this level, because the small size of the plant provides little buffering for fluctuation in operation; moreover, such facilities often have limited resources and limited access to the expertise needed to solve specific operational problems. For these small facilities, 0.2 mg/L or less is a practicable level for aluminum in finished water [7]. Different Al(III)-based coagulants such as poly-aluminum-silicate-chloride (PASiC) [8], polyaluminum chloride [9–11], and flocculants [12] have been studied to avoid the elevated RA concentrations in water. The Italian Official Journal set 1 and 2 mg/L of Al(III) as the limits in sewer and receiving water discharge endpoints, respectively [13].

Numerous studies have dealt with the effect of Al(III) on different biota, including freshwater, marine, and terrestrial organisms. Table 1 summarizes various effects of aluminum to different species. Atlantic salmon (*Salmo salar*) was exposed to environmentally realistic concentrations of inorganic monomeric Al(III) only (<15 μM) and Al(III) mixtures, and sub-lethal concentration of zinc (1.7 to 0.8 μM) at the pH levels 4.5, 4.8, and 5.2. For Al(III)-only exposure, the LC_{50} (M) value exhibited a marked pH dependence (higher values at low pH). The presence of zinc reduced survival times in the Al+Zn mixtures [36]. The alum-treated water exerted mutagenicity in the presence of fulvic acid in water [37]. An 80 μM of Al(III) caused rapid changes in cytoplasmic organization of vegetative filaments in the coenocytic algae, *Vaucheria longicaulis* var. *macounii* [38]. A recent study also evaluated the toxicity of alum and its mixture with an anionic polymer on *Daphnia magna* and *Selenastrum capricornutum* [39]. Alstad et al. [40] observed only a very slight ameliorating effect increased in brown trout (*Salmo trutta* L.) due to ionic strength. Among marine organisms, sea urchin and mussel embryos exhibited Al(III)- and Fe(III)-associated developmental toxicity, either as complex mixtures or as Al(III) or Fe(III) salts at 10^{-6} to 10^{-5} M concentrations [26,27]. Al-tolerant cultivar TAM202 and Al-sensitive cultivar TAM105 of winter wheat (*Triticum aestivum* L.)

Table 1
Main toxicity endpoints associated to Al(III) exposures

Organisms/testing objects	Exposure routes	Endpoints	Endpoints
Humans	Renal dialysis	Neuro-, myelo-	[14,15]
	Antacid drugs	Bone and nephrotoxicity	[16–20]
Mammals	Oral or parenteral	Developmental defects	
Mammalian	Medium	Neurofilamentous changes	[21]
Cells		Excess ROS formation	[22]
		Membrane lipoperoxidation	[23]
Other vertebrates		Developmental defects	
Birds	Yolk sac		[24]
Frogs	Medium		[25]
Invertebrates	Medium	Developmental defects	
Sea urchins		Cytogentic abnormalities	[26–28]
Bivalves		Transmissible offspring damage	[29,30]
		Changes in ROS formation	[31,32]
Plants	Medium	Phytotoxicity	
		Cytologic and cytogenetic anomalies	[33,34]
		Gene induction (of cytoskeletal proteins)	[35]

were exposed to Al(III) (up to 150 μM) and it was observed that TAM105 absorbed more Al(III) than TAM202 and its root growth (length) was inhibited more severely [41]. Yousef [42] showed that $AlCl_3$ significantly induced free radical formation, and decreased the activity of glutathione S-transferase and the levels of sulfhydryl groups in rabbit plasma, brain, testes, and kidney. In biological systems, Al(III) is deemed to complex with oxygen donor ligands, especially phosphates, and it was reported to do so in soils, in the gastrointestinal tract, and in cells. The presence of humic acid at neutral pH partly reduced toxicity on freshwater snail, *Lymnaea stagnalis*, and had no effect on the level of Al accumulation in tissues; thus, humic acid may play an important role in limiting Al toxicity to freshwater organisms [43]. In a recent study, secondary-treated effluent was submitted to coagulation using alum and alum-treated samples were found to exhibit toxicity in freshwater snail, *L. stagnalis*, at neutral pH [43].

Coagulation process using inorganic coagulants has been approved to improve the quality of secondary-treated municipal wastewater treatment plant (MWWT). The removal efficiency of coagulation process was proved by adding aluminum sulfate. The effluent of ferric chloride supported coagulation process was toxic to *Vibrio fisheri* bacteria and exhibited mutagenic effects on strain TA 98; these effects were increased during coagulation with ferric chloride (both in absence and presence of flocculant). However, the addition of aluminum coagulants resulted in a decrease in mutagenic potential of secondary effluents, due to the extended removal of organic matter [44]. Toxicity of coagulated leather tannery wastewater was first reported to be toxic to sea urchin embryos [45]. A similar approach confirmed that sea urchins were to use toxicity assessment of leather tannery effluents collected from different points of leather tannery wastewater treatment plant [46].

Due to the lack of data on the assessment of MWWTP effluents treated by coagulation process, the present study was carried out on the MWWT to evaluate the efficacy of alum coagulation for complying with the COD (<160 mg/L) and TSS (80 mg/L) discharge standards [13]. Raw wastewater was coagulated in the laboratory using 150 and 450 mg/L of alum, and with or without Prodefloc® anionic polymer at pH ranging from 5 to 7. The supernatants were analyzed for COD, TSS, and RA content, and tested by sea urchin bioassays to meet the following specifications: (a) acute and/or developmental toxicity, (b) changes in fertilization success, and (c) cytogenetic abnormalities. The RA concentrations were evaluated regarding ΔAl (Al in raw wastewater—Al in Jar test supernatants), alum dose, and pH used in coagulation process, raw water COD content, and toxicity findings. The study provided a set of results pointing to an increase in wastewater toxicity following alum-based coagulation.

2. Materials and methods

2.1. Sampling location and collection

The MWWTP located in Eastern Naples receives a 30,000 m^3/d inflow and the plant utilizes a coagulation–flocculation process with 200 mg/L $FeSO_4$ and 60 mg/L of $Ca(OH)_2$, and with final chlorination. Effluent toxicity on *D. magna* was previously reported for the same treatment plant [47]. Two raw wastewater samples (S1 and S2) were collected from inflow after the coarse screen is tested for toxicity; Jar test experiments and effluents of the existing treatment plant were taken after final chlorination process to test toxicity. The first sample was taken to characterize wastewater and accordingly to define the alum dose compared to literature. As well its RA content was analyzed. The samples were refrigerated (4˚C) in ice boxes and delivered to the laboratory in 1 h. The samples were tested and collected in maximum 2 h for toxicity and they were refrigerated at 4˚C for subsequent experiments to be run within a week after collection.

2.2. Coagulation experiments

Jar tests experiments were performed on raw S2 sample. S2 was coagulated using $Al_2(SO_4)_3{\cdot}18H_2O$ (alum) at levels of 150 and 450 mg/L and at pH ranging from 5 to 7 according to the literature [44–47]. The samples were stirred at 100 rpm for 1 min, at 30 rpm for 20 min, and then were kept for 30 min before testing [47]. Prodefloc® anionic polymer (2 mg/L), which is currently used in the MWWTP, was added. Then S2 alum-coagulated supernatants were collected to be submitted to COD, TSS, and RA measurements.

2.3. Chemical analyses

Raw and coagulated wastewater samples were analyzed for their COD, TSS, and aluminum contents according to standard methods [48]. Samples were filtered using 0.45 μm GF/C Whatman filter papers. Residues of Al(III) were measured by flame atomic absorption spectrophotometry (Varian spectraAA 10 Plus, USA).

2.4. Sea urchins

Sea urchins from the species *Sphaerechinus granularis* were collected in the Bay of Naples, Italy, (kindly provided by the "Anton Dohrn" Zoological Station, Naples) during the study period of 2000/2001, and the results belonging to the project were released for publication during 2009–2010. The approach and continuous research in the field of toxicological methods to establish Water Frame Directive in the EU water resources was pioneering step for the attempt to publish the findings presented hereby. Gametes were obtained and embryo cultures were run as described previously by Pagano et al. (1986) [26]. Controls acted as untreated negative control (filtered seawater, FSW) and 2.5×10^{-4} M $CdSO_4$ acted as positive control [26,27]. Test samples (S2 and S2-alum coagulated supernatants) were suspended in FSW at concentrations of 1% v/v. The exposure of embryos (~20–30 embryos/ml) occurred throughout development from zygote (10 min after fertilization) up to the pluteus larval stage (72 h after fertilization).

Sperm bioassays were conducted on sperm cell suspensions, by a standard exposure of a 0.2% suspension of concentrated sperm pellet for 10 min [26,27]. Thereafter, 0.5% supernatant sperm was used to inseminate untreated egg suspensions (50 to 100 eggs/mL). Changes in the fertilization success of exposed sperm were determined by scoring the percent of fertilized eggs in fresh cleaving embryos (1 to 3 h post-fertilization). The observations of larvae were performed on living plutei (n = 100 for each replicate) immobilized in 10^{-4} M chromium sulfate approximately 72 h after fertilization [26]. The following outcomes were evaluated: (i) retarded (R) plutei [≤1/2 size *vs.* normal (N) plutei]; (ii) pathologic (P1) malformed plutei; (iii) pathologic embryos (P2) that were unable to differentiate up to the pluteus larval stage, and (iv) dead (D) embryos/larvae [scored as dead plutei (D1), or early dead embryos (D2)]. All observations were carried out double blind by trained readers, each evaluating a complete set of readings.

Cytogenetic analysis was carried out on 30 cleaving embryos from each of four replicate cultures, fixed in Carnoy's fluid 5 h after fertilization, then stained by acetocarmine and rinsed in 20% acetic acid. The parameters being analyzed included quantitative and morphological abnormalities. Quantitative abnormalities were: (a) mean number of mitoses per embryo (MPE); (b) percent interphase embryos (% IE), lacking active mitoses, and (c) metaphase/anaphase ratio (M/A). The morphological abnormalities were scored as: (i) anaphase bridges; (ii) lagging chromosomes; (iii) acentric fragments; (iv) scattered chromosomes, and (v) multipolar spindles. These abnormalities were both scored individually and reported as total mitotic aberrations per embryo; the percentage of embryos having ≥1 mitotic aberrations [% E(Ab+)] was also scored according to Pagano et al. [27].

2.5. Statistical evaluation

Comparisons among coagulation pH, alum dose, and influent COD and TSS values regarding coagulation efficiency and percentage developmental defects in sea urchin larvae (P1+P2 classes), percentage of fertilized eggs were tested for significance using multiple factor ANOVA followed by the multiple comparison test of Tukey (Honest Significant Difference, HSD) using STATISTICA Software [49]. Significance level of statistical analyses was always set at α = 0.05.

3. Results and discussion

3.1. Jar test results

As reported in Table 2, Jar test removal efficiencies of COD and TSS parameters for S2 were >50 and 60, respectively. Optimum COD removal was

Table 2
Characteristics raw wastewater (S1, S2) and Jar-test effluents using alum (S2-alum-colagulated) for the samples from the MWWTP (100 rpm for 1 min, 30 rpm for 20 min, 30 min for settling at room temperature, Prodefloc® anionic polymer of 2 mg/L)

Sample no.	Raw wastewater samples			Alum-coagulated Jar test supernatants					
				(COD removal %)			(TSS removal %)		
	COD (mg/L)	TSS (mg/L)	Al-SO_4* (mg/L)	pH = 5.0	pH = 6.0	pH = 7.0	pH = 5.0	pH = 6.0	pH = 7.0
S1	330	70	–						
S2	460	100	150*	58	73	83	60	80	70
	460	100	450*	84	72	86	65	80	85

*Amount added to raw wastewater.

obtained at pH 7.0 while for the highest TSS removal was observed at pH 6.0. Increasing alum dose from 150 to 450 mg/L improved both COD and TSS removal. A significant COD removal increase was obtained at 5.0 pH (form 58 to 84%) while the highest TSS removal (form 70 to 85%) increase was observed at 7.0 pH. This non-dose-dependent variation in COD and TSS removal should be due to influent wastewater characteristics which influence the coagulation efficiency. However, no statistically significant (p>0.05) relation between influent characteristics and pH-related coagulation efficiency was observed.

The RA levels varied in the S2 alum-coagulated supernatants (Table 3). A non-significant increase in RA concentration *vs.* alum dose was observed at 5.0 pH (p = 0.424) (Fig. 1(a)). As shown in Fig. 1(b), coagulation pH affected significantly RA concentrations (p = 0.012). Further performed Tukey test also showed that RA concentration was divided in two groups *vs.* pH (5.0, p = 0.015 and 6–7.0, p = 0.016). On the other hand, neither alum dose and coagulation pH did show any significant relationship on removal efficiency of COD (p = 0.383 for alum dose and p = 0.492 for pH) and TSS (p = 0.270 for alum dose and p = 0.140 for pH).

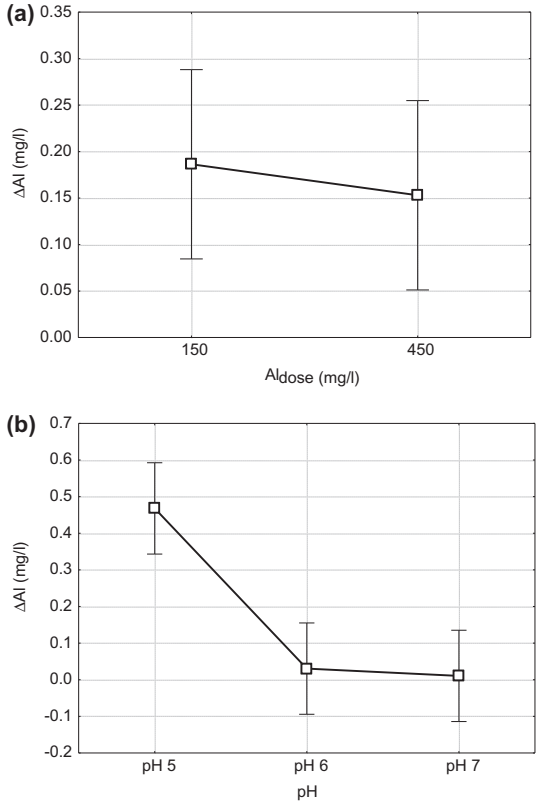

Fig. 1. The relation between Al out (RA) and Al dose (a) and ΔAl (raw wastewater-coagulated wastewater) vs. pH (b) in S2 related samples (Jar test conditions and abbreviations are as indicated in Table 1).

3.2. Sea urchin bioassays

When *S. granularis* embryos were reared in 1% S1 and chlorination-treated effluent of the MWWTP to monitor the effect of $FeSO_4$ currently used in the coagulation process in the plant, no significant increase in developmental defects was observed (data not shown). However, during a second sampling, (S2) chlorinated effluent of the treatment plant was more severely toxic (P2) than raw wastewater. By exposing developing embryos to 1% S2 alum-coagulated supernatants (150 and 450 mg/L, pH ranging 5–7), a significant increase in larval malformations (P1) and developmental arrest (P2) was observed, to the highest extent following coagulation using 450 mg/L at pH 6.0 and 150 mg/L of alum at pH 7.0 (Fig. 2).

Table 3
Residual Al (III) (RA) concentrations in S2 and S2 alum-coagulated supernatants (Jar test conditions and abbreviations are as indicated in Table 1)

Al-SO_4 dose added to the sample (mg/L)	Al (III) in raw S2 (mg/L)	RA concentrations in S2 alum-coagulated supernatants (mg/L)		
		pH = 5.0	pH = 6.0	pH = 7.0
150	0.008	0.51	0.02	0.005
450	0.008	0.41	0.025	0

Fertilization success of *S. granularis* sperm was not affected by exposure to 1% S2, with a slight, non-significant decrease at 5.0 and 6.0 pH values (Fig. 3).

The cytogenetic analysis of *S. granularis* embryos reared in 1% S2 and S2 alum-coagulated supernatants failed to show any mitotoxic effect (decrease in mean number of mitoses per embryo, MPE). However, an increase in the metaphase to anaphase (M/A) ratio, and in percent mitotic aberrations (%Ab+) was both exerted by S2 and S2 alum coagulated wastewater at pH ranging from 5 to 7.0 (Table 4).

3.3. Discussion

The Jar test experiments provided evidence that coagulation process provides sufficient COD removal efficiency in the MWWTP at an alum dose of

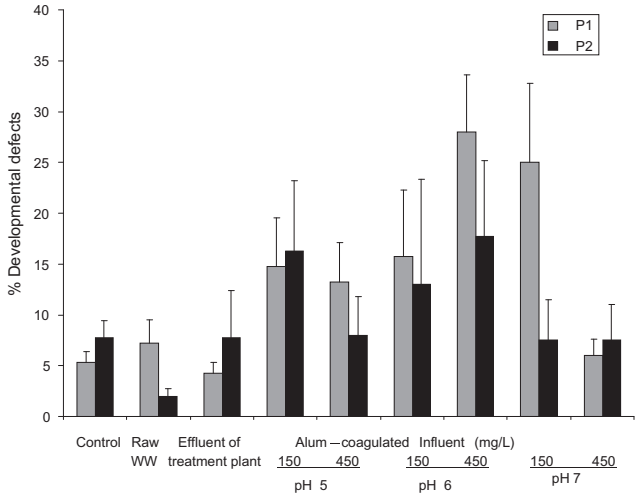

Fig. 2. Percent developmental defects in *S. granularis* larvae reared 72 h in S2 and at S2 alum-coagulated supernatants at different pH using 150 mg/L of alum. Quintuplicate experiment (mean ± SE). Abbreviations: larval malformations (P1); developmental arrest (P2) (Jar test conditions and abbreviations are as indicated in Table 1).

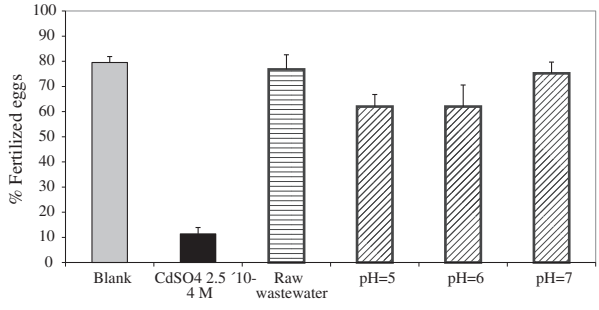

Fig. 3. Fertilization success rate of *S. granularis* sperm reared in S2 and S2 alum-coagulated supernatants at different pH using 150 mg/L of alum. 12-replicate experiments (mean ± SE) (Jar test conditions and abbreviations are as indicated in Table 1).

150 mg/L meeting the discharge standards (COD<160 mg/L) in agreement with our previous results [47]. RA concentrations were significantly influenced by different (raw wastewater-coagulated wastewater aluminum concentrations) aluminum concentrations as well as alum dose used in coagulation process while no relationship among coagulation pH, RA, and influent COD was observed. This result could be due to coagulation pH ranging between 5 and 7, i.e. over 4 to influence the RA concentrations, in particular its most toxic species [4].

A 1% S2 alum-coagulated supernatants resulted in a dramatic increase of developmental defects in sea urchin larvae. These data confirm our previous findings of toxicity due to aluminum and iron coagulation to sea urchins [27,45]. The rates of developmental defects appeared to vary with respect to the pH values utilized in Jar tests suggesting a pH-dependent release of toxic Al(III) species in this complex mixture. The spermiotoxic results failed to show any significant effects of alum-coagulated wastewater in the present study. The cytogenetic analysis of the embryos reared in wastewater samples (raw or alum-coagulated) failed to show any effect in terms of mitotoxicity, while the metaphase: anaphase ratio and percentage mitotic aberrations showed suggestive, yet not significant increases in embryos reared in influent following alum coagulation [27]. The toxicity outcomes were different due to pH values and alum dose used in coagulation. A concentration-related toxicity for alum dose in coagulation had been observed by Guida et al. [39] in *D. magna*, yet the present results failed to show any significant concentration-related embryotoxicity in sea urchin embryos at the alum levels being tested.

Together, the results highlighted the raw wastewater characteristics on developmental toxicity, such as pH-related Al(III) speciation, and alum complex formation in wastewater. The results and findings obtained in this study are expected to contribute to municipal wastewater management and will raise the question to evaluate alum-coagulation as double-ended sword for environmental pollution control. However, this study is to be applied on

Table 4
Cytogenetic analysis of *S. granularis* embryos exposed to 1% S2 and S2 alum-coagulated supernatants. Twelve-replicate experiments (mean ± SE) (Jar test conditions and abbreviations are as indicated in Table 1, MPE: mean number of mitoses per embryo; M/A: metaphase/anaphase ratio; [% *E*(Ab+)]: the percentage of embryos having ≥1 mitotic aberrations; mean ± standard error, SE)

Samples	Number of repeats	MPE	M/A	[% *E*(Ab+)]
Untreated controls (Blank)	15	5.7 ± 1.1	2.2 ± 1.1	5.6 ± 5.9
Raw S2	5	6.4 ± 2.3	1.4 ± 0.5	10.0 ± 7.1
S2 alum-coagulated (pH range 5–7)	20	6.3 ± 1.5	1.0 ± 0.5	9.8 ± 6.2

different wastewater characteristics by the use of multi-species tests for evaluating different endpoints to assess the impact of alum-coagulation in the environment.

4. Conclusion

The results provide evidence for the influence of alum-based coagulation on domestic wastewater, resulting in a limited efficiency regarding COD, TSS, and RA concentrations. Sea urchin bioassays allowed us to detect developmental toxicity providing evidence for a definite influence of pH on coagulation mixture, attributed to Al(III) speciation.

In conclusion, wastewater characteristics altogether may account for the different responses to Jar tests in the sea urchin embryotoxicity bioassay. The prospect of utilizing sea urchin bioassays in testing wastewater toxicity may be suggested on a broader scale and supported by chemical analysis in order to explain aluminum chemistry in wastewater and in marine environment.

Unconfined to freshwater biota, the quality of domestic wastewater should be evaluated in terms of toxicity to marine organisms, for the potential impact of inland wastewater in confined marine water bodies. The fate of alum-coagulated effluent must be detailed for marine water which is crucial for the understanding of aluminum chemistry.

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