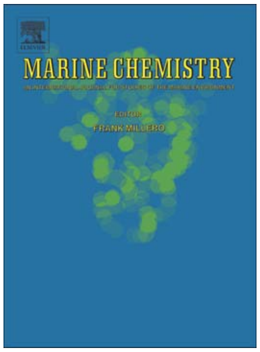

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Influence of trace metal release from volcanic ash on growth of *Thalassiosira pseudonana* and *Emiliania huxleyi*.

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Abstract

Recent studies demonstrate that volcanic ash has the potential to increase phytoplankton biomass in the open ocean. However, besides fertilizing trace metals such as Fe, volcanic ash contains a variety of potentially toxic metals such as Cd, Cu, Pb, and Zn. Especially in coastal regions closer to the volcanic eruption, where ash depositions can be very high, toxic effects are possible. Here we present the first results of laboratory experiments, showing that trace metal release from different volcanic materials can have both fertilizing and toxic effects on marine phytoplankton in natural coastal seawater. The diatom *Thalassiosira pseudonana* generally showed higher growth rates in seawater that was in short contact with volcanic ash compared to the controls without ash addition. In contrast to that, the addition of volcanic ash had either no effect or significantly decreased the growth rate of the coccolithophoride *Emiliania huxleyi*. It was not possible to attribute the effects to single trace metals, however, our results suggest that Mn plays an important role in regulating the antagonistic and synergistic effects of the different trace metals. This study shows that volcanic ash can lead to changes in the phytoplankton species composition in the high fall-out area of the surface ocean.

Key words: *Thalassiosira pseudonana*, *Emiliania huxleyi*, volcanic ash, pumice, fertilization, toxicity

Introduction:

Large volcanic eruptions can eject enormous amounts of ash and pumice far into the open ocean (Olgun et al., 2011, and references therein). The importance of volcanic ash as a source of nutrients and trace metals in the ocean, however, has been largely overlooked compared to the much better studied effects of desert dust inputs (Jickells et al., 2005). This is mainly because of the unpredictability of volcanic eruptions and the difficulties associated with sampling of uncontaminated and unhydrated volcanic ash material. Therefore, direct measurements of the effect of volcanic ash on marine phytoplankton in the field are very rare. The first trace metal analysis within the ash fall-out area of a volcanic eruption (Etna, Sicily, 2001) demonstrates both strongly elevated trace metal concentrations (e.g. > 600 nM Fe, >20 nM Cu, and > 150 nM Mn in surface waters) and higher chlorophyll values compared to previous years (Censi et al., 2010). Also, there is growing evidence from satellite data that phytoplankton productivity is increased with volcanic ash deposition in the open ocean (Duggen et al., 2007; Hamme et al., 2010; Langmann et al., 2010; Lin et al., 2011; Uematsu et al., 2004). A main factor suggested for this is fertilization with iron (Duggen et al., 2010), which limits phytoplankton productivity in large regions of the world's ocean, but macronutrients could have significant effects as well in oligotrophic regions (Uematsu et al., 2004).

Besides iron, volcanic ash releases the macronutrients NH_4^+, NO_3^-, PO_4^{3-}, and SiO_2; volatile compounds such as Br^-, Cl^-, F^-, SO_4^{2-}; and a variety of trace metals such as Al, Cd, Co, Cu, Mn, Ni, Pb, and Zn (Duggen et al., 2007; Frogner Kockum et al., 2006; Frogner et al., 2001; Jones and Gislason, 2008; Witham et al., 2005). Some of the trace metals, especially Cd, Cu, Pb, and Zn, have potentially toxic effects on marine phytoplankton in higher concentrations (Brand et al., 1986; Sunda, 1988). Toxic effects of Cu from desert dust have recently been shown in incubation experiments (Paytan et al., 2009), however, the degree of Cu toxicity from this source in the open ocean remains questionable (Sholkovitz et al., 2010). Since the amount of volcanic ash deposition into seawater is strongly dependent on the distance from the volcano, coastal areas will receive higher trace metal inputs with so far unpredicted effects for their phytoplankton community structure. Most studies addressing the combined effects of different trace metals for marine phytoplankton have been performed in laboratory experiments using trace metal chelators such as EDTA to control free trace metal concentrations (e.g. Sunda and Huntsman, 1983; Sunda and Huntsman, 1996; Sunda and Huntsman, 1998b; Sunda and Huntsman, 2000; Tortell and Price, 1996). Further, the main focus of these studies was to mimic low trace metal availability as in open ocean regions. Therefore, our understanding of antagonistic and synergistic effects of trace metals in coastal regions is still very limited today.

In this study we investigate the trace metal release of four volcanic ashes and one pumice sample from different regions of the Pacific Ring of Fire and their effect on the growth rate and Cu ligand production of the diatom *Thalassiosira pseudonana* and the coccolithophoride *Emiliania huxleyi* in natural coastal seawater.

Material and Methods:

Throughout the experiment trace metal clean techniques were used to prevent trace metal contamination. All materials that came in contact with the ash, the cultures, or the media were acid cleaned before use. Sample and culture handling took place inside a class 100 clean bench.

Volcanic ash and pumice samples:

All volcanic ashes were sampled as clean as possible shortly after explosive eruptions and never had contact with rain or seawater before the experiments. The pumice material was collected by breaking a bigger piece of pumice in two and carefully crushing the inside to get material that was least affected by erosion. The location and year of the eruptions, as well as the collection sides are described in table 1. The ashes and crushed pumice were stored double bagged in zip lock bags in a dry environment. The samples are unsieved and had no contact with metal-containing materials.

Cultures:

Axenic cultures of *Thalassiosira pseudonana* and *Emiliania huxleyi* were obtained from the Provasoli-Guillard Center for Culture of Marine Phytoplankton, Bigelow Laboratories. The cultures were grown at 18 °C and 100 µmol quanta m $^{-2}$ s^{-1} at a light : dark cycle of 16 : 8 hours. Before the experiments, both cultures were grown for at least 8 generations in natural coastal seawater enriched with nitrate, phosphate, silicate, and vitamins in f/2 concentrations (*T. pseudonana)* and in f/20 concentrations without silicate additions (*E. huxleyi).* The trace metal mix was prepared without EDTA and added 1/500 times more diluted compared to the original f/2 recipe. To ensure that no EDTA was left in the media, the cultures were transferred into fresh, EDTA free media several times until EDTA concentrations were diluted to values below 1 pM.

Growth experiments:

For every experiment, 2.67 g ash were added to 1 L natural seawater and gently shaken for 10 minutes by hand, followed by a 5 minutes settling time. This ash concentration corresponds to a ~ 6.6 cm thick sediment ash layer as calculated by Duggen et al. (2007). This is a realistic ash layer thickness for sediments in coastal regions near active volcanoes as modeled by Olgun et al. (2011) and reported from sediment cores of the Northwest Pacific by Cao et al. (1995). No or only a low decrease in pH of maximum 0.1 units was observed after the ash addition.

Afterwards, the ash was removed through in-line sterile filtration using 0.22 µm, Millipore, Sterivex units. This water was mixed in different ratios with sterile filtered natural seawater without any nutrient or trace metal addition to gain four concentrations corresponding to contact with 0.53, 1.07, 1.6, and 2.67 g ash l^{-1}. The controls were grown in natural seawater without ash or trace metal addition. Thereafter, the macronutrients were added in f/2 concentrations for *T. pseudonana* incubations and in f/20 concentrations for *E. huxleyi* incubations. No trace metal solutions were added to the ash incubations or the controls. The cultures were added at cell densities of about 200 cells ml^{-1} for *T. pseudonana* and 300 cells ml^{-1} for *E. huxleyi* and incubated in triplicates for 9 days. Samples for cell numbers were taken every third day and immediately measured without addition of preservatives using a Flow Cytometer (Becton Dickinson FACSCalibur). Specific growth rates (μ d^{-1}) were calculated from the least-squares regression of cell counts versus time during exponential growth. For statistical analyses Students t-tests were used. Differences found are reported as significant in the text if $p<0.05$.

Ligand experiment:

In a second experiment, we tested the effect of Sakura-jima ash and Apoyeque pumice additions on the Cu ligand production of *T. pseudonana* and *E. huxleyi*. 2 g ash or pumice material, respectively, were given to 1 L of the same seawater as used for the growth experiment and treated as described above. The cultures were grown in triplicates. Since we only measured cell densities at day one and 9 in this experiment we did not calculate growth rates.

The copper complexing ligand concentrations were determined using cathodic stripping voltammetry after Campos and van den Berg (1994). Seawater samples were filtered through in-line sterile filtration using 0.22 µm, Millipore, Sterivex units and stored frozen until analysis. The samples were slowly thawed in a fridge and aliquots of 10 ml each were given to Teflon voltammetric cells. 50 µl borate buffer and 20 µl SA (0.01 M) as well as copper standard at increasing concentration from 0 – 180 nM were added to each sample. The solutions were equilibrated over night.

Trace metal concentrations:

The trace metal concentration of the seawater before and after 15 minutes of contact with the volcanic material was measured in direct determination after 10 times dilution in MilliQ using a HR-ICP-MS (Thermo Finnigan Element) as described in Ardelan et al. (2009). All samples (10 ml) were filtered through a 0.22 µm syringe top filter (Millipore Sterivex) and acidified with 20 µl HNO_3 (FLUKA, trace select).

Results:

Trace metal release

Volcanic ash samples generally mobilized significant amounts of trace metals after short (15 minutes) contact with seawater. However, the trace metal release of the four ash samples and the pumice showed significant variations. From all four ashes tested, Al, Mn, and Zn were released in very high concentrations between 17.4 nM and 1749.7 nM (table 2). Cu and Fe were released in concentrations of 10.2 nM and 2.1 nM, and 10.8 nM and 5.7 nM from the Arenal and the Rabaul-Tavurvur volcanoes, respectively. For the ashes from the Popocatepetl and the Sakura-jima volcanoes, Fe release exceeded Cu release with 7.0 nM and 83.2 nM compared to 4.4 nM and 5.6 nM. Cd, Co, Ni, and Pb were released in concentrations ≤ 1.5 nM from the Arenal, Popocatepetl, and the Rabaul-Tavurvur ashes. The ash from the Sakura-jima volcano generally released the highest trace metal concentrations of the four ashes tested except for Al, Cu, and Zn. The concentrations for Cd, Co, and Mn released from this ash were about 20 to 45 times higher compared to the other ashes while Fe and Ni were about 10 times higher compared to the other ashes.

In contrast, the Apoyeque pumice material released relatively low concentrations of trace metals within 15 minutes contact time. No release of the metals Cd, Pb and Zn was detected. Fe and Mn were the trace metals released in the highest concentrations with 3.9 nM and 5.9 nM, respectively.

Phytoplankton growth

In most cases the addition of volcanic ash significantly changed the growth rate of *T. pseudonana* and *E. huxleyi*. Interestingly, the ashes tested had an opposite effect on both species (fig. 1). *T. pseudonana* had a growth rate between 0.37 d^{-1} and 0.5 d^{-1} in the controls. The addition of medium ash concentrations between 0.53 and 1.6 g l^{-1} significantly increased the growth rate to values between 0.53 d^{-1} and 0.65 d^{-1} (p values between 0.004 and 0.04, respectively) for the ashes Popocatepetl, Rabaul-Tavurvur, and Sakura-jima. In contrast, the ash of the Arenal volcano significantly (p values between 0.002 and 0.007) decreased the growth rate d^{-1} of this species at medium ash concentrations. Here, the growth rates were 0.17 d^{-1}, 0.23 d^{-1}, and 0.15 d^{-1}, respectively. At the highest ash concentrations added (2.67 g l^{-1}) the growth rate of *T. pseudonana* was significantly higher compared to the controls for the three ashes Arenal (μ=1.15 d^{-1}; p=0.03), Popocatepetl (μ=0.75 d^{-1}, p=0.007), and Sakura-jima (μ=1.0 d^{-1}, p=0.001). Only the incubation with the ash from the Rabaul-Tavurvur volcano did not show a significant difference at the highest ash concentration compared to the control. The growth rates in the controls without ash addition were between 0.64 d^{-1} and 0.96 d^{-1} for *E. huxleyi*. The incubations with different ash concentrations from the Arenal, Popocatepetl, and Rabaul-Tavurvur eruptions did not show any significant difference compared to the respective controls. However, the Sakura-jima ash significantly decreased the growth rate of this species at every concentration tested. Here, growth rates decreased from 0.76 d^{-1} in the controls down to 0.07 d^{-1} at the highest ash concentration (p values between 0.03 and 0.002).

Ligand experiment

Compared to the control, *T. pseudonana* again showed a much higher growth with addition of the Sakura-jima ash (fig. 2). After 9 days of incubation the cell numbers were 67.5 times higher in the ash treatment. Addition of pumice material still increased the growth rate of this species and final cell numbers were 6.8 times higher compared to the control.

Like in the first experiment, ash from the Sakura-jima eruption decreased the growth of *E. huxleyi*, which did not grow at all in the ash treatment of this experiment. However, addition of pumice material increased the growth of *E. huxleyi* with final cell numbers being 5.5 times higher compared to the controls.

Possible toxic effects for marine phytoplankton species as a result of Cu released from volcanic material are suggested in the literature (Duggen et al., 2007; Jones and Gislason, 2008). The production of organic, Cu binding ligands is a known defense mechanism at elevated Cu concentrations for some phytoplankton species (Croot et al., 2000; Leal et al., 1999) and was therefore tested in this study.

The copper ligand concentration in the natural seawater without any additions was 3.5 ± 1.1 nM. Right after the addition of volcanic ash or pumice material to the seawater, the ligand titrations became very noisy and the concentration measured varied between 2.5 and 22.1 nM. Most likely surface complexation of colloids leaching from the volcanic materials interfered with the measurements. These colloids may be nanoparticles disaggregating from the ash on contact with the water, or it may be colloidal secondary phases precipitating either before or after filtration, e.g. iron oxyhydroxides. Similar problems have been observed in glacial meltwaters in Iceland containing large concentrations of volcanic ash (M. Bau pers. comm.). These very variable ligand concentrations in the beginning of the experiment make it difficult to determine any changes during the course of the incubations (figure 3) and we did not find a production of Cu binding ligands in any of the incubations with the Sakura-jima ash. However, our data show a strong production of about 60 nM of Cu binding ligands by *T. pseudonana* in the incubations with the Apoyeque pumice material (figure 3).

Discussion:

The Cd, Co, Cu, Fe, Ni, Pb, and Zn concentrations in the coastal seawater used as culture media were close to those reported for other coastal regions (Kozelka and Bruland, 1998; Kuma et al., 1998; Lares et al., 2009; Öztürk et al., 2002; Wells et al., 1998).

Since trace metal concentrations in coastal regions are much higher compared to the open ocean, trace metal additions would usually not be expected to have significant effects here. However, Öztürk et al. (2002) show that phytoplankton growth can also be limited by iron in coastal regions. The bioavailability of iron in natural systems is dependent on the chemical form of iron rather than on the total concentration. In coastal systems a large fraction of iron is colloidal Fe, which releases bioavailable iron much slower compared to dissolved labile Fe(III) organic complexes (Kuma et al., 1995). Moreover, high concentrations of organic matter can also reduce Fe bioavailability in coastal waters (Öztürk et al., 2002). Therefore, addition of iron could possibly have beneficial effects even in high-metal coastal systems. Further, addition of trace metals can in some cases alter toxicity effects of other metals as in the case of Mn. Since Cd, Cu, Zn and Mn compete for the same uptake systems in *T. pseudonana*, high Mn concentrations inhibit the uptake of these trace metals and prevent toxicity (Sunda et al., 1981; Sunda and Huntsman, 1996; Sunda and Huntsman, 1998a; Sunda and Huntsman, 1998b; Sunda and Huntsman, 1998c). Rather than the absolute free Cd, Cu, and Zn concentration, it is the ratio of Mn to these metals which determines their toxicity. In other words, input of Mn to polluted, high Cd, Cu, and Zn waters could quench toxic effects of these metals for phytoplankton and increase growth.

Fertilizing effects of volcanic material for *T. pseudonana*:

In our study, *T. pseudonana* had a growth rate between 0.37 d^{-1} and 0.5 d^{-1} in the controls, which is clearly below the maximum growth rate of this species reported for artificial media (about 1.7 d^{-1} (e.g. Ellwood and Hunter, 2000; Price et al., 1987; Sunda and Huntsman, 1992)). This indicates that the coastal seawater either did not supply this species with sufficient micronutrients or that the concentration of toxic metals was high enough to depress growth of this species. It is possible that the high Zn and Cu concentrations in the controls (279.4 and 6.3 nM, table 2) suppressed growth of this species since the Mn concentrations were relatively low (3.1 nM, table 2). In laboratory experiments, *T. pseudonana* only showed a distinct reduction in growth rate to about 40 % of the maximum growth rate at much higher free Cu concentrations of 10 µM (Brand et al., 1986). However, in Brand et al.'s experiment (1986) all other trace metals, including Mn, were buffered by EDTA to levels ideal for phytoplankton growth, excluding the possibility of antagonistic and/or synergistic effects of different trace metals. Toxic effects of Cu for *T. pseudonana* can be reached at much lower concentrations when Mn concentrations are suboptimal (Sunda and Huntsman, 1983). Another possibility could be that the growth of *T. pseudonana* in the controls is limited by the relatively low Co concentrations (0.5 nM table 2). Other than *E. huxleyi, T. pseudonana* is known to have Co requirements which can not be met by Zn (Sunda and Huntsman, 1995; Whitfield, 2001).

The general increase in growth rate of the diatom *T. pseudonana* with addition of the volcanic ash could be caused by a combination of Mn, Fe, and Co fertilization. It is noticeable that the ash that released the highest concentration of Mn, Fe, and Co and the lowest concentrations of Cu (Sakura-jima) had the strongest effect on the growth rate of *T. pseudonana* (fig. 1; table 2). A toxic effect of Cu could be the reason for the observed decrease in growth rate at medium ash concentrations of the Arenal volcano, which released the lowest concentrations of Mn, Fe, and Co and the second highest concentrations of Cu (10.2 nM g^{-1}). It is however noticeable that this ash had a significantly positive effect on growth of *T. pseudonana* at the highest ash concentration used, while the Rabaul-Tavurvur ash with similar Cu concentrations (10.8 nM g^{-1}) and higher Mn concentrations did not have a positive effect on growth of this species at the highest ash concentrations. This shows that other factors influencing the growth of *T. pseudonana* must be involved as well.

The input of potentially toxic trace metals such as Cd and Cu from the Rabaul-Tavurvur, the Popocatepetl and the Sakura-jima ashes did not seem to have a significant effect on this species. This is most likely because they are buffered by the high input of Mn from these ashes. Since the Mn release from the volcanic materials tested here was always much higher compared to the combined Cu and Cd release (table 2) it is likely that this would quench a possible Cd and Cu toxicity for diatoms in the field in general. A recent evidence of volcanic ash fertilization in coastal waters was shown in the Ionian coast during the 2001 eruption of Mount Etna (Censi et al., 2010). Here, similar effects could be responsible for the reported increase in chlorophyll as in particular the dissolved concentrations of Mn, Fe, and Co increased more compared to other metals (Censi et al., 2010).

A surprising result of this study is the strong production of Cu binding ligands by *T. pseudonana* in the incubations with Apoyeque pumice (figure 3). As the pumice released less Cu than any of the ashes tested, it is unlikely that this ligand production was a Cu detoxification mechanism. Many organic ligands are not metal specific and therefore the Cu binding organic substance could have been produced for other reasons.

Toxic effects for *E. huxleyi*:

The maximum growth rate for *E. huxleyi* reported in the literature is about 1.1 d^{-1} (Zondervan, 2007) which is close to the maximum growth rate we observed in the controls without ash addition (0.96 ± 0.04 d^{-1}, figure 1). Since *E. huxleyi* can meet its Co requirements partly by replacing it with Zn (Sunda and Huntsman, 1995; Whitfield, 2001) it is possible that the high Zn concentrations in the controls are beneficial to this species. Also, *E. huxleyi* showed to be very tolerant to Cu toxicity and only reduced its growth rate to about 80% at free Cu concentrations of 20 µM in medium buffered with 0.1 µM EDTA (Brand et al., 1986) and above 48 nM in culture experiments without addition of EDTA (Leal et al., 1999), which is higher than the Cu concentrations in any of our ash addition treatments. The highest Cu concentration used here was 35.1 nM in the treatment with the highest concentration of Rabaul-Tavurvur ash (10.8 nM Cu released per g ash * 2.67 g ash L^{-1} + 6.3 nM Cu in the background water, table 2).

In contrast to *T. pseudonana*, addition of volcanic material did not have any significant effect (Arenal, Popocatepetl and Rabaul-Tavurvur ash, figure 1) or resulted in a significant decrease of the growth rate of *E. huxleyi* (Sakura-jima ash, figure 1). The only volcanic material that increased the growth of this species compared to the controls was the pumice from the Apoyeque volcano, which released trace metals in much lower concentration compared to the other ashes tested (table 2). In both incubations with the Sakura-jima ash we observed a strong toxic effect for *E. huxleyi* despite the high Fe and Mn concentrations released from the ash (figure 1 and 2). We can only speculate what caused this effect but direct toxic effects of Cu, Cd, Pb, or Zn seem implausible for the following reasons:

E. huxleyi is known to produce Cu binding ligands when grown under Cu stress (Leal et al., 1999) but we did not find any production of Cu binding ligands by *E. huxleyi* above the high variance when incubated with the Sakura-jima ash (figure 3). Further, the Cu and Zn concentrations released from the Sakura-jima ash are lower compared to the other ashes and *E. huxleyi* has a high Cu tolerance (Brand et al., 1986; Leal et al., 1999). Cd and Pb are released in higher concentrations from the Sakura-jima ash compared to the others, however, similar to Cu, *E. huxleyi* showed to be very tolerant to Cd compared to other species tested (including *T. pseudonana*) and only reduced its growth rates by 50 % at free Cd concentrations of 580 nM in the NTA buffered media used (Payne and Price, 1999), and at 1 µM free Cd in EDTA buffered media, respectively (Brand et al., 1986). Further, Pb and Cd additions of up to 25 nM each to coastal seawater without the addition of EDTA only showed a low toxic effect on *E. huxleyi* and reduced its growth rate by about 13% (Vasconcelos and Leal, 2001).

Other than for *T. pseudonana*, the extremely high Mn concentrations released by the Sakurajima ash could be one reason for the negative effects on growth of *E. huxleyi*. It is known that trace metal uptake systems for Cd, Co, and Zn differ substantially between *E. huxleyi* and marine diatoms (Sunda and Huntsman, 2000). Similarly, the Mn uptake system and its importance for preventing the uptake of toxic metals could be different in *E. huxleyi* as well, but there are not published data on this so far. From our experiments it seems that in *E. huxleyi* Mn does not prevent the uptake of toxic metals or is toxic itself in these high concentrations. This theory is supported by the observation that the addition of Apoyeque pumice, which released the lowest Mn concentration of all volcanic materials tested, was the only one that significantly increased the growth of *E. huxleyi* (figure 2).

Conclusions:

Our experiment shows that the same volcanic ash can both, increase and decrease growth rates of different phytoplankton species, which will ultimately lead to changes in species composition with implications for community productivity and export of the affected ocean regions. Other than suggested for open ocean regions, however, it seems that the inputs of Fe and Cu through volcanic eruptions are not the main factors influencing phytoplankton growth and species composition in coastal, high trace metal regions. Rather, the complex interactions of antagonistic and synergistic effects between different trace metals and especially the Mn concentration seem to control phytoplankton growth in our experiments. If similar effects could also apply for the open ocean are difficult to predict. However, it is likely that the overall effects of volcanic eruptions for the marine ecosystem are much more complex than only the previously suggested Fe fertilization and Cu toxicity.

Despite the enormous amount of work in this field, most studies investigating combined effects of different trace metals for marine phytoplankton have been performed in EDTA buffered media using mainly the diatom species T. *oceanica, T. weissflogii*, and *T.*

pseudonana, and the coccolithophoride *E. huxleyi*. We know very little about combined trace metal interactions and their effects for other marine phytoplankton species in natural seawater. To be able to better understand implications of combined natural trace metal inputs such as volcanic eruptions or also desert dust storms for natural phytoplankton communities and their effects for ecosystem productivity we urgently need more detailed studies addressing synergistic and antagonistic effects of trace metals under more natural culture conditions without the addition of metal chelators in both coastal and open ocean regions.

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Table 1: Origin of the volcanic material, year of eruption, and description of sampling site.

Volcano, location	Eruption year	Collection distance	Description
Arenal, Costa Rica	1993	At the foot of the volcano	Silty-fine sandy
Popocatepetl, Mexico	2000	18.7 km NW from crater	Silty
Rabaul-Tavurvur, Papua New Guinea	2002	At the foot of the volcano	Fine-sandy
Sakura-jima, Japan	2007	3 km NW of the Minamidake crater	Silty
Apoyeque, Nicaragua	1.9 ka	570 km from volcano	Crushed pumice

Table 2: Trace metal concentrations (in nM) of the coastal seawater used as growth medium and the concentrations released by 1 g (calculated from the 2.67 g added to 1 L) of the different ashes and the pumice material after 15 minutes of contact with seawater for the growth and the ligand experiment. Please note: In this study, 0.53, 1.07, 1.6, and 2.67 g ash L^{-1} were used. Therefore, the final trace metal concentrations in the incubations are the concentrations given in this table times the ash concentration used plus the background concentration of the seawater.

growth experiment		Al	Cd	Co	Cu	Fe	Mn	Ni	Pb	Zn
background	seawater	44.5	0.5	0.5	6.3	15.1	3.1	8.5	0.2	279.4
released per 1 g material	Arenal ash	1749.7	0.0	0.1	10.2	2.1	17.4	0.5	0.0	77.5
	Popocatepetl ash	77.6	0.2	0.4	4.4	7.0	29.5	1.5	0.1	119.7
	Rabaul-Tavurvur ash	330.9	0.1	1.0	10.8	5.7	69.5	1.3	0.0	58.0
	Sakura-jima ash	1377.6	4.2	33.0	5.6	83.2	1298.6	13.9	0.8	34.0
ligand experiment		**Al**	**Cd**	**Co**	**Cu**	**Fe**	**Mn**	**Ni**	**Pb**	**Zn**
background	seawater	78.3	0.6	0.1	6.4	22.6	1.7	8.4	0.01	158.9
released per 1 g material	Apoyeque pumice	1.5	0.0	0.2	1.3	3.9	5.9	1.2	0.0	0.0
	Sakura-jima ash	1105.0	3.8	31.4	9.0	50.6	1237.3	9.9	0.6	39.1

Figure captions:

Figure 1: Growth rate d^{-1} of *T. pseudonana* and *E. huxleyi* in the controls and in incubations with 0.53, 1.07, 1.6, and 2.67 g ash L^{-1} added. All incubations are done in triplicates, error bars denote standard deviation. If not visible, error bars are smaller than symbols. Controls were grown in natural seawater without ash or trace metal additions; macronutrients were added to all incubations.

Figure 2: Cell numbers of *T. pseudonana* and *E. huxleyi* in the controls and with addition of 2 g L^{-1} Sakura-jima ash and Apoyeque pumice over nine days. All measurements are done in triplicates, error bars denote standard deviation. If not visible, error bars are smaller than symbols. Controls were grown in natural seawater without ash or trace metal additions; macronutrients were added to all incubations.

Figure 3: Changes in Cu ligand concentration after the 9 days of incubation in the controls and with addition of 2 g L^{-1} Sakura-jima ash and Apoyeque pumice material compared to the start. All measurements are done in triplicates, error bars denote standard deviation. Controls were grown in natural seawater without ash or trace metal additions; macronutrients were added to all incubations.

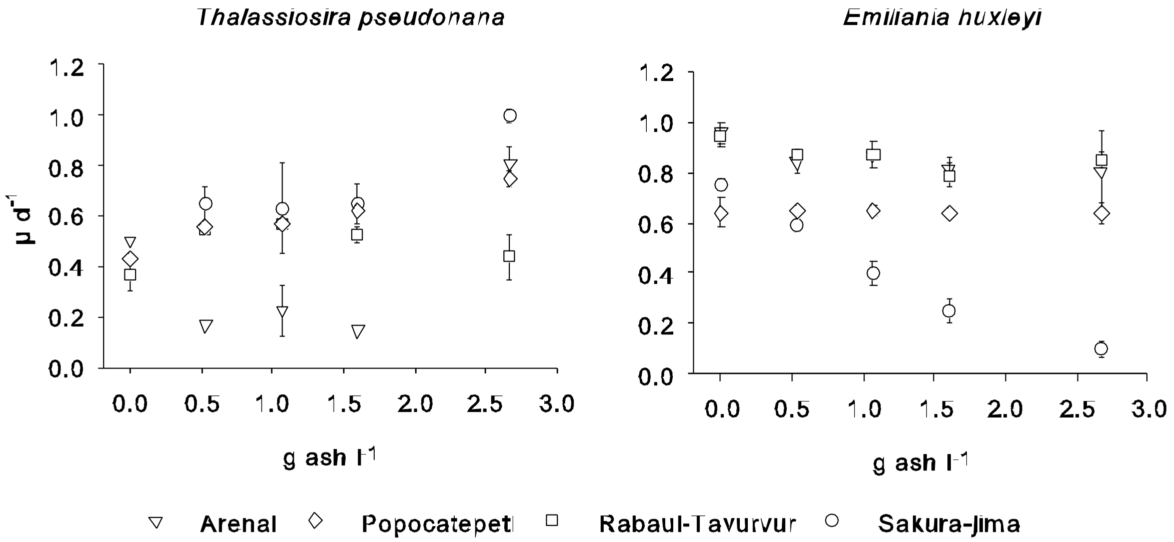
Thalassiosira pseudonana
Emiliania huxleyi
μ d-1
1.2
1.0
0.8
0.6
0.4
0.2
0.0
0.0
0.5
1.0
1.5
2.0
2.5
3.0
g ash l-1
▽ Arenal
◇ Popocatepetl
□ Rabaul-Tavurvur
○ Sakura-Jima

Fig. 1

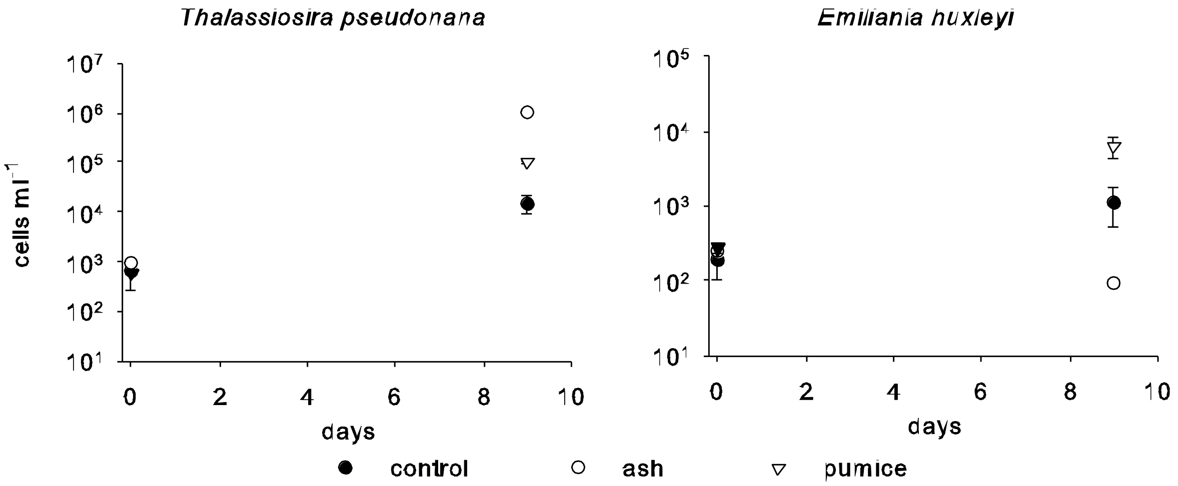
Thalassiosira pseudonana
Emiliania huxleyi
cells ml-1
10⁷
10⁶
10⁵
10⁴
10³
10²
10¹
0
2
4
6
8
10
days
● control
○ ash
▽ pumice

Fig. 2

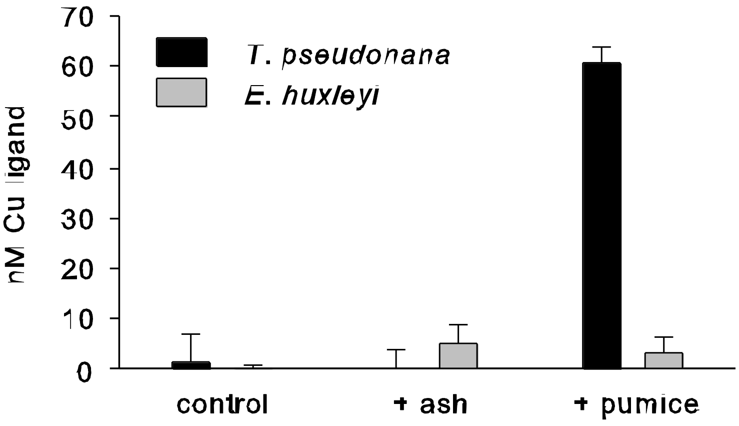
70
60
50
40
30
20
10
0
nM Cu ligand
T. pseudonana
E. huxleyi
control
+ ash
+ pumice

Fig. 3

Highlights

- We tested the effect of volcanic ash on growth of *T. pseudonana* and *E. huxleyi*
- Volcanic ash increased growth of *T. pseudonana* but not of *E. huxleyi*
- Mn seems important to regulate the effects of different trace metals from the ash
- Volcanic eruptions have the potential to change phytoplankton community structures